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THE LESIONS IN EXPERIMENTAL AMEBIC DYSENTERY *

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In the development of knowledge of the pathologic changes in amebic dysentery as it occurs in the human being as well as in the experimental animal, few differences of opinion have arisen concerning the character of the lesions produced, particularly the advanced lesions of the bowel, which are recognized and described as ulcerations. The earliest careful description of this more advanced lesion varies but little from that by the most recent investigators. True, the early investigators were largely interested in the ulcerative processes of a disease that had just been recognized as having a new and specific etiology, and they studied and described largely the lesions that are now looked on as typical of amebic dysentery, namely, the sharply punched-out ulcer with elevated edges and undermined base, the "shirt-button" ulcer of the French. Concerning this lesion as it appears macroscopically and microscopically, there has been little controversy.

The same agreement does not prevail, however, in the attempts to explain the ability of *Endameba histolytica* to produce ulceration of the intestine. Here, speculation and opinion based on controversial evidence have had to be substituted for exact knowledge. Is *Endameba histolytica* always pathogenic? Does it act by the secretion of a cytolytic or proteolytic toxin or ferment, and thus loosen or destroy the cells that stand in the way of its entrance into the tissue, as thought by Dopter,[1] or does it gain entrance into the wall of the bowel by virtue of its ability to force its way between and among the cells in its path, as taught by Schaudinn?[2] Is the primary attack on the submucosa of the bowel, as believed by Councilman and Lafleur,[3] and if so, how is this tissue reached without injury to the overlying mucosa? Or is the first lesion

* Submitted for publication, March 12, 1930.

1. Dopter, C.: Sur quelques points relatifs à l'action pathogène de l'amibe dysentérique, Ann. de l'Inst. Pasteur **19**:417, 1905.

2. Schaudinn, Fritz: Untersuchungen über die Fortpflanzung einiger Rhizopoden, Arb. a. d. k. Gsndhtsamte. **19**:547, 1903.

3. Councilman, W. T., and Lafleur, H. A.: Amoebic Dysentery, Johns Hopkins Hosp. Rep. **2**:395, 1891.

that of the mucosa,[4] and if so, does it first invade the mucosal crypts (Wenyon,[5] Kofoid[6]), or does it attack the surface of the mucosa, destroying epithelial and interstitial cells with equal facility (Dopter,[4] Christoffersen, and James[7])?

It is evident that answers to these questions cannot be found by study of the fully developed intestinal lesions, and more recent descriptions have included smaller and presumably earlier lesions, in the interpretation of which there is, again, difference of opinion. It also seems unlikely that the nature and exact site of the primary attack on the part of the ameba could be determined from the evidence presented by human material alone. Intestinal postmortem changes occur with great rapidity, especially in the colon, and the motility of the ameba is such that a delay of not more than an hour in obtaining the material for study might permit the occurrence of such alterations that the lesions present would fail to represent the conditions prevailing during life.

Difficulties in procuring earlier and unchanged intestinal ulcers of man led to study of the lesions in the more readily controlled experimental animals. Artificial production of amebic dysentery can be effected more or less at will; the time of onset can be readily determined, and the lesions for study can be procured before postmortem changes have essentially modified their appearance.

Unfortunately, artificially induced amebiasis, and a study of the lesions produced in the intestinal tract of the cat, the animal most frequently used in experiments, although they contributed to knowledge of the disease, had failed, up to the time the study reported in this paper was made, to answer definitely the questions concerning the nature and the earliest point of attack of *Endameba histolytica,* even so far as the cat is concerned. The intestinal ulcers of the cat suffering from amebic dysentery are similar to those of man ill with the same disease. This similarity has given rise to a tendency to interpret the lesions of man in the light of those of the experimentally infected animal. Difference in the disease as found in man clinically and in the

4. Dopter, C.: Anatomie pathologique de la dysentérie amibienne, Arch. de méd. expér. et d'anat. path. **19**:505, 1907. Christoffersen, D. R.: Zur pathologischen Anatomie der Amöbendysenterie, Virchows Arch. f. path. Anat. **223**:350, 1917. Kaufmann, Eduard: Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte, Berlin, W. de Gruyter & Company, 1922, vol. 1, p. 608.

5. Wenyon, C. M.: Experimental Amoebic Dysentery and Liver Abscess in Cats, London School of Tropical Medicine **2**:27, 1912-1913.

6. Kofoid, C. A.: Amoeba and Man, Univ. Calif. Chronicle 1923, pp. 149 and 291.

7. James, quoted by Harris, H. F.: Virchows Arch. f. path. Anat. **166**:67, 1901.

experimental animal, as well as in the lesions presented by both, makes hazardous the unreserved application to one of them of conclusions obtained from a study of the other.

Knowing that the amebic lesions of the bowel of the experimentally infected cat closely resemble those found in man ill with the same disease, but recognizing at the same time, certain significant differences that distinguish the lesions in one from those in the other, I believed that carefully conducted work with the artificially infected cat might throw light on the nature of the initial attack of *Endameba histolytica* on the intestine. To this end, a series of experiments was undertaken, the nature and results of which are here presented. All kinds of experimental lesions were examined, but special attention was devoted to the early lesion. If the answer to this query is to be found experimentally, it should come out of the earliest lesion that still may be recognized as amebic.

In the beginning of this experimental work, the first material suitable for inoculation into animals came at a time when no animal adapted to the needs of the experiment was available. The attempt to maintain this material for later use led to the artificial cultivation of *Endameba histolytica,* with which study 1 of this paper is concerned. Study 2 deals with the production of amebic dysentery in kittens, both by amebas artificially maintained and by amebas obtained from the stools of persons infected with *Endameba histolytica* and of kittens ill with amebic dysentery. Study 3 is concerned with the intestinal lesions thus produced.

1. CULTURAL METHODS

DATA FROM THE LITERATURE

In view of later developments in the cultivation of the parasitic amebas of man, it now seems probable that Cutler [8] succeeded in growing *Endameba histolytica* as early as 1918, although his work was not confirmed by others at the time, and Dobell [9] apparently did not consider his work successful. His medium was such that it might have succeeded, and Boeck and Drbohlav [10] were able to grow *Endameba histolytica* on it, although poorly (see appendix to study 1). It is probable that Yoshida [11] kept the ameba of dysentery alive in his medium (see appen-

8. Cutler, D. W.: A Method for the Cultivation of Entamoeba Histolytica, J. Path. & Bact. **22**:22, 1918.

9. Dobell, Clifford: The Amoebae Living in Man. A Zoological Monograph, London, John Bale Sons & Danielsson, Ltd., 1919, pp. 34 and 58.

10. Boeck, W. C., and Drbohlav, J.: The Cultivation of Endamoeba Histolytica, Am. J. Hyg. **5**: 371, 1925.

11. Yoshida, Kazuyoshi: The Encystment of Dysentery Amebae in Vitro, J. Exper. Med. **28**:387, 1918.

dix), although it is hard to explain the temperature at which they survived for from thirty-six to seventy-two hours. No attempts were made by him to make subcultures from the strains that he used.

Of the numerous attempts to cultivate the amebas parasitic in animals and man, the first to be generally accepted as a success was the cultivation of an endameba from the turtle by Barret and Smith [12] in 1924. The medium was composed of human blood serum, 1 part, and 0.5 per cent solution of sodium chloride, 9 parts. The growth of the endameba was successful at both room and icebox temperature, although some strains were lost through the early overgrowth of bacteria and *Blastocystis*. When the strains were established, transfers were required every two or three days at room temperature and once a week at icebox temperature. Five strains were thus grown, ranging in age from several weeks to nineteen months at the time of the first report. A later report by the same authors [13] told of the successful cultivation of *Endameba ranarum* for more than eight months. Taliaferro and Holmes [14] compared the amebas from Barret and Smith's cultures with those of the turtle from which they were grown and succeeded in cultivating them on Loeffler's dehydrated blood serum, 1 part, and 0.5 per cent solution of sodium chloride, 10 parts.

Previous to this time, many workers besides Cutler and Yoshida had attempted cultivation of the intestinal amebas parasitic in man. Many mediums had been used, some with apparent success, even to the extent that amebas grown on them produced dysentery in experimental animals. In spite of apparently confirmatory inoculation in animals, it is now generally believed that no one, with the possible exception of one or two observers, succeeded in growing parasitic amebas. Rather it is thought that the organisms so readily grown were contaminants from the locality in which the experiments were made or were free-living amebas accidentally ingested by the patient and on their way through the intestinal tract.

In 1924, the same year of Barret and Smith's work with the ameba of the turtle, Boeck and Drbohlav [15] read a paper before the meeting of the American Society of Tropical Medicine. The following year this

12. Barret, H. P., and Smith, N. M.: The Cultivation of an Entamoeba from the Turtle, Chelydra Serpentina, Am. J. Hyg. **4**:155, 1924.

13. Barret, H. P., and Smith, N. M.: The Cultivation of Endamoeba Ranarum, Ann. Trop. Med. **20**:85, 1926.

14. Taliaferro, W. H., and Holmes, F. O.: Endamoeba Barreti, N.Sp., from the Turtle, Chelydra Serpentina; a Description of the Amoeba from the Vertebrate Host and from Barret and Smith's Cultures, Am. J. Hyg. **4**:160, 1924.

15. Boeck, W. C., and Drbohlav, J.: The Cultivation of a Pathogenic Amoeba (Entamoeba Histolytica) from a Case of Relapsed Amoebic Dysentery, Am. J. Trop. Med. **4**:440, 1924.

work was published in full.[10] Two mediums were used by them with apparently equal success: Locke's egg-serum (L. E. S.) medium, and Locke's egg-albumin (L. E. A.) medium (see appendix). Each consisted of a Dorsett egg-slant overlaid with inactivated human serum and Locke's solution in the proportion of 1:8, or with 1 per cent crystallized egg albumin in Locke's solution. On these mediums, they grew two strains of *Endameba histolytica* that showed, culturally, the essential characteristics of the pathogenic parasite, and that, on inoculation into kittens, produced clinical dysentery with the amebas in the stools and also the typical ulceration of amebic dysentery with the amebas in the lesions. This work was further confirmed within the same year by Andrews,[16] Kofoid and Wagener,[17] Drbohlav,[18] Das Gupta,[19] Guérin and Pons,[20] Thomson and Robertson,[21] and later by many other workers.

Because mediums of Boeck and Drbohlav have been found successful in the cultivation of the parasitic amebas of man, most of the subsequent work has been done with one medium, or with modifications of mediums that seemed to promise superior cultural properties. As originally prepared, the hydrogen ion concentration of the mediums varied from 7.2 to 7.8, but this was quickly reduced as a result of the rapid multiplication of intestinal bacteria that are invariable contaminants of every protozoal culture made directly from the stool. This necessitated the making of frequent subcultures. A buffer was added by Boeck and Drbohlav [10] without result, and later, a buffer and starch were added by Drbohlav [22] working alone. He substituted Ringer's solution for Locke's solution and replaced the egg-slant with a slanted N. N. N. medium as the underlying solid portion. He found that the medium, thus modified, kept the organisms alive for from ten to twenty-four days. Kofoid and

16. Andrews, J. M.: The Cultivation of Endamoeba Histolytica by Boeck's Method, Am. J. Hyg. **5**:556, 1925.

17. Kofoid, C. A., and Wagener, Edna H.: The Behavior of Endamoeba Dysenteriae in Mixed Cultures with Bacteria, and Studies of the Effects of Certain Drugs upon Endamoeba Dysenteriae in Vitro, Univ. Calif. Pub. Zool. **28**:127, 1925.

18. Drbohlav, Jaroslav: Présentation d'amébes dysentériques et cultures, Bull. Soc. path. exot. **18**:121, 1925; Une nouvelle prevue de la possibilité de cultiver Entamoeba dysenteriae type histolytica, Ann. de parasitol. **3**:349, 1925; Culture d'Entamoeba dysenteriae type tetragena minuta, ibid. **3**:358, 1925; Cultures d'Entamoeba coli Loesch, 1875, Emend. Schaudinn 1903, ibid. **3**:364, 1925.

19. Das Gupta, B. M.: A Note on the Cultivation of an Entamoeba from a Monkey (Macacus Rhesus), Indian M. Gaz. **60**:323, 1925.

20. Guérin, F. H., and Pons, R.: Culture d'Entamoeba dysenteriae par le procédé de W. C. Boeck et Jaroslav Drbohlav, Bull. Soc. path. exot. **18**:517, 1925.

21. Thomson, J. G., and Robertson, Andrew: Notes on the Cultivation of Certain Amoebae and Flagellates of Man, Using the Technique of Boeck and Drbohlav, J. Trop. Med. **28**:345, 1925.

22. Drbohlav (footnote 18, second reference).

Wagener [17] found that a reduction of the dextrose in Locke's solution from 2.5 Gm., as used by Boeck and Drbohlav,[10] to 0.25 Gm. gave a more prolonged growth. As a cover fluid, they used the whites of two eggs to 1,000 cc. of the modified Locke's solution and adjusted the mixture with dilute hydrochloric acid to a hydrogen ion concentration of 7.6 or 7.8. In an attempt to inhibit bacterial growth, various substances, including acriflavine, were added to the medium without result. In three cases of active amebic dysentery in which cultures were made by them, growth was positive in all and was carried to the fifty-fifth, the sixty-second and the eightieth transplants, respectively. Subcultures were made every forty-eight hours, but by renewing the cover fluid every day, survival of amebas in one tube, for as long as nine days, occurred. With but one exception, the cultures of motile forms from six cases of chronic dysentery failed to show any amebas at the end of twenty-four hours. One attempt to cultivate cysts failed.

Although Drbohlav [22] substituted an unnamed starch for sugar in the cover fluid, it remained for Dobell and Laidlaw [23] to work out the relative values of the various starches for this purpose. Of these, the best was rice starch, which they found to be readily ingested by the amebas, and the mediums thus modified gave the most luxurious and prolonged growth observed by them up to that time (see appendix). They found, also, that the addition of acriflavine in the proportion of 1:20,000 for several generations inhibited bacterial growth and promoted richness of the amebic cultures. Organisms were grown on this medium as follows: four strains of *Endameba histolytica*, two from men and two from monkeys; one strain of *Endameba gingivalis*; two strains of *Endameba coli*, one from man and one from monkey, and one strain of *Endameba nana* from man and monkeys. Kofoid and Wagener [24] announced that the addition of defibrinated blood to Locke's solution as the overlying fluid gave good results, and that bacteria grew less rapidly in this than in the preparations that contained human serum. Of the various bloods thus used, the best was that from the rabbit, used in 0.5 per cent concentration. St. John [25] made use of the cultural method in the diagnosis of dysentery and found it more favorable than examination of the direct smear. In his work, the solid egg base was used, whereas the overlying fluid consisted of human serum or horse serum, 1 part, to Locke's solution, 7 parts. In the latter, the sugar was

23. Dobell, Clifford; and Laidlaw, P. P.: On the Cultivation of Entamoeba Histolytica and Some Other Entozoic Amoebae, Parasitology **18**:283, 1926.

24. Kofoid, C. A., and Wagener, Edna H.: A Simplified Medium for the Cultivation of Endameba Dysenteriae, J. Lab. & Clin. Med. **11**:683, 1926.

25. St. John, J. H.: Practical Value of Examination for Endameba Histolytica by Culture, J. A. M. A. **86**:1272, 1926.

reduced to 0.1 per cent only. In three cases, cysts from stools sent by mail gave positive cultural results.

Craig [26] reported the successful cultivation of *Endameba histolytica* in a medium consisting only of Locke's solution, 7 parts, and inactivated human serum, horse serum or rabbit serum, 1 part. The best of these was inactivated human serum. In this medium, *Endameba histolytica* remained alive and mobile for eleven days, and successful transfers were made at the end of eight days. Transfers made every twenty-four to forty-eight hours continued the culture for three months. Later, Craig [27] announced the successful cultivation of *Endameba histolytica* in a medium consisting of inactivated human serum, 1 part, and Ringer's solution, 7 parts, and in inactivated human serum and physiologic solution of sodium chloride in the same proportions. In the latter, he kept one strain alive for six weeks, with forty transfers in this time. He found that Ringer's solution and solution of sodium chloride both inhibited bacterial growth, and that the amebas grown on these mediums were larger and more active than those grown on the egg mediums. Craig and St. John,[28] using these simplified mediums in the diagnosis of amebic dysentery by cultural methods, found that Locke's solution and serum, Ringer's solution and serum, and physiologic solution of sodium chloride and serum were equally good, and that all were better than Locke's egg serum of Boeck and Drbohlav for this purpose.

Laidlaw, Dobell and Bishop [29] left the egg base out of their medium and kept the fluid portion of the original composition: horse serum, 1 part, and Ringer's solution, 8 parts, with the addition of a small amount of sterile rice starch (see appendix). As a buffer, there was later added to Ringer's solution 0.2 Gm. of disodium hydrogen phosphate for each 100 cc. In the latter medium, amebic growth lasted for from ten days to two weeks, although transfers were made every week. Four strains of *Endameba histolytica* were successfully grown on this culture medium: one from man isolated three years before by Drbohlav and three from monkeys, one of the strains from monkeys being eighteen months old, one seven months old and one recently isolated.

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26. Craig, C. F.: A Simplified Method for the Cultivation of Endamoeba Histolytica, Am. J. Trop. Med. **6**:333, 1926.

27. Craig, C. F.: Observations upon the Cultivation of Endamoeba Histolytica, Am. J. Trop. Med. **6**:461, 1926.

28. Craig, C. F., and St. John, J. H.: The Value of Cultural Methods in Surveys for Parasitic Amebae of Man, Am. J. Trop. Med. **7**:39, 1927.

29. Laidlaw, P. P.; Dobell, Clifford, and Bishop, A.: Further Experiments on the Action of Emetine in Cultures of Entamoeba Histolytica, Parasitology **20**: 207, 1928.

In the literature on the cultivation of parasitic amebas of man up to 1928, the impression obtained is that the artificial cultivation of parasitic amebas is comparatively simple, and that this may be accomplished on a rather wide variety of mediums. This view is particularly strengthened by the work of Craig and St. John,[25] who used the simplest mediums successfully in the diagnosis of dysentery by cultural methods. With the exception of Kofoid and Wagener,[17] who failed to grow the ameba from six cases of chronic dysentery, and of Cutler, whose work is not generally accepted, there are only a few communications dealing with the difficulties of growing parasitic amebas. The first is that of Miller,[30] who reported in 1928 that the use of Boeck and Drbohlav's medium in nineteen cases of amebic dysentery had given survival of amebas for longer than one day in two cases only. He felt that there was some promise in the medium as modified by Dobell by the addition of starch, as with this preparation he had one strain of *Endameba histolytica* surviving in four successive subcultures. Johns [31] said that the original Boeck's medium, modified by the addition of rice starch and acriflavine, 1:30,000, was the only medium that cultural survival of amebas for any length of time. In a study of the value of cultural methods in the examination of stools for protozoal parasites, as compared with direct examination of stools, Magath and Ward [32] found agreement in the case of *Endameba histolytica* in only 47 per cent of cases and 42 per cent of stools examined. They mentioned an "almost universal failure encountered in an attempt to transfer any of these cultures."

METHODS AND RESULTS

That *Endameba histolytica* might at all times be available for experiments on animals, it seemed wiser to attempt their cultivation than to depend for material on the uncertain and irregular appearance of patients who have the organism in their stools. This attempt resulted in a series of experiments and observations concerning the growth characteristics of *Endameba histolytica* that appears to be of interest and value.

The material used in the attempted cultivation of *Endameba histolytica* was obtained from eight patients who presented themselves at the Mayo Clinic for examination and treatment during the year 1928 (table 1). Cultivation of positive stools was not attempted as a routine;

30. Miller, M. W.: Difficulty in Cultivation of Endamoeba Histolytica, Proc. Soc. Exper. Biol. & Med. **25**:762, 1928.

31. Johns, F. M.: In a personal communication to T. B. Magath, in 1928.

32. Magath, T. B., and Ward, C. B.: Laboratory Methods of Diagnosing Amoebiasis, Am. J. Hyg. **8**:840, 1928.

neither was there a selection of only those cases in which there was evidence of active amebic dysentery with pronounced evidence from an examination of the stools. Of the eight patients from whose stools cultures were made, only five had moderately active symptoms, with stools varying from an early morning looseness to as many as fourteen stools a day. On inspection of the gross specimens, some of these stools contained mucus and blood. Three patients were free from intestinal symptoms at the time of examination, and one who was in the hospital with an amebic abscess of the liver did not give a history of previous dysentery or diarrhea. In one patient who did not have

TABLE 1.—*Data Concerning Cases Which Furnished the Material for Experiments in the Cultivation of Amebas; Results of Cultivation*

Case	Age, Yr.	Sex	Where Contracted	Duration of Symptoms	Stools According to History	Cultural Strain	Growth	Comment
1	27	M	Venezuela	1 year	3 to 6 a day, with mucus and blood	A	+	
2	23	M	Indiana	3 months	Formed; no history of dysentery	D	—	Amebic abscess of liver
3	36	M	Texas	7 months	Formed	E F	+ +	Three months later, abscess of liver
4	48	F	India	2 years	Formed	G	+	
5	48	F	China	24 years	5 to 12 each day; watery	H	+	
6	22	F	Minnesota	2 years	Early morning looseness	I	+	
7	44	M	Indiana	2 years	3 to 4 each day; mixed with urine	J	+	
8	27	M	Illinois	2 years	5 to 14, with mucus and blood; no salts previous to examination	K L	— +	

intestinal symptoms, an amebic abscess of the liver developed later. From the stools of these eight patients, ten cultures were made, and growth occurred for one day or more in eight, an incidence of 80 per cent. Of the eight positive growths, only three survived culturally longer than six days, or four generations, whereas one grew one day only and did not appear in the first subculture. Of the three that grew the longer time, it seems reasonable to suppose that all might, with care, have been continued indefinitely, as one was lost through accident after having grown seventy-two days, and two were abandoned at the end of one hundred one and sixteen days, respectively, the purpose for which they were grown having been accomplished.

The culture showing the poorest growth (strain J) which might, in fact, have been a survival only, was made from a stool that was mixed with urine (table 2).

Of the two cultural attempts that failed, one culture (strain D) was made from cysts from the solid stool of a patient (case 2) ill at the time with an amebic abscess of the liver. The other culture (strain K) was the first of two attempts to grow *Endameba histolytica* from the stool of a patient (case 8) who was having from five to fourteen typical dysenteric stools a day. In this instance, there was some delay in preparing the culture, and the stool, which was small, not more than 30 cc., became cold before being used. The next day, from the same patient a stool that was cultivated at once gave a positive growth (strain L) that was continued for sixteen days.

Of factors influencing the successful cultivation of *Endameba histolytica* from stools, it is possible that the taking of Epsom salts may be an inhibiting factor, as suggested by Craig.[33] In the examination

TABLE 2.—*The Artificial Cultivation of Endameba Histolytica from Man*

Strain	Source Case	Material	Growth	Generations	Days of Growth	Results of Inoculation of Animals	Comment
A	1	Motile forms	+	30	72		
D	2	Cysts	—	—	—		
E	3	Motile forms	+	1	2		
F	3	Motile forms	+	4	6		
G	4	Motile forms	+	1	2		
H	5	Motile forms	+	44	101	+	Abandoned
I	6	Motile forms	+	2	4	—	
J	7	Motile forms	+	1	1		
K	8	Motile forms	—	—	—		
L	8	Motile forms	+	8	16	+	Abandoned

of stools for parasites, the routine procedure at the Mayo Clinic calls for a preliminary dose of salts, that the material to be examined may be fluid and may contain the motile forms on which the diagnosis is made, rather than the cysts. This rule may be departed from at times in the case of a patient giving a history of diarrhea. Only one of the patients (case 8) whose stools were used for cultural purposes did not receive salts. Consequently, the effect of a preliminary dose of salts in the inhibition or prevention of amebic growth, culturally, cannot be determined from this series.

In handling my cultures, no attempt was made to determine the thermal death point of the amebas or their resistance to deleterious influences; rather, every effort was made to protect them from factors that might jeopardize their continued survival. To this end, cultures were first planted as early as possible, while the stools from which the cultures were made were still fresh and warm. The tubes of mediums to be inoculated, both those for the first cultures and those for all subsequent transplants, were warmed to approximately 37 C.

33. Craig, C. F.: In a personal communication to T. B. Magath.

before the inoculations were made. As soon as possible after inoculation, the tubes were placed in the incubator, which was kept at 37 C., and each time one of them was removed for any purpose, it was kept out a limited length of time and was stood, during that interval, in a container of warm water.

For the removal of culture material for examination or for subinoculation, a capillary pipet was used. In the observations of Boeck, Kofoid and others, the amebas were found in the egg slant cultures at the lower point of juncture between the slant and the glass of the tube. Dobell said that in his modified mediums the amebas were found in the starch that had gravitated to the lower part of the tube or in the groove between the solid portion of the medium and the wall of the tube. As here determined, unless the starch was added only in minute quantities, the amebas were found on the surface of this collection and not in its interior. This fact may help to explain a negative reading one day, followed later by a positive reading in the same tube.

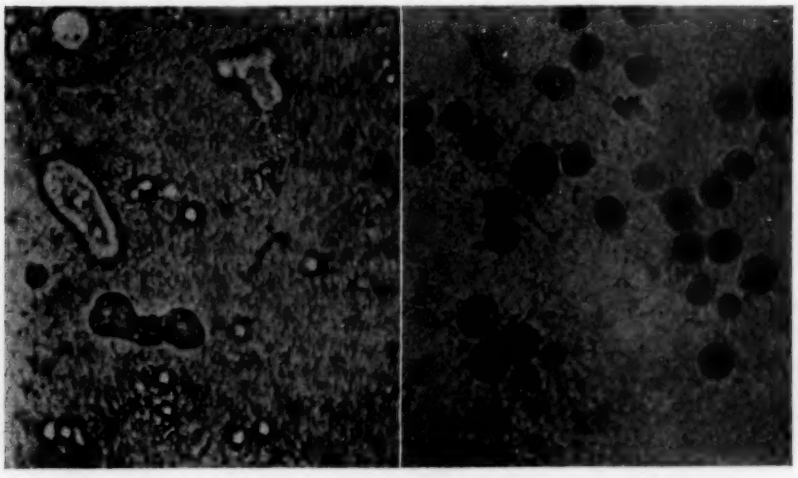
Subcultures from growing cultures were made at the height of growth, and this varied somewhat with each culture strain. The average interval was forty-eight hours, although this was sometimes exceeded and but rarely reduced. With every strain grown, most careful selection was made of the tube or tubes from which all subcultures were made; in all cases, the tube that showed the most prolific growth was selected. Nor were the old tubes discarded until they were found negative. Several times it was possible to prolong the growth only by going back to an older generation for cultural material, the younger having given negative results.

In the cultivation of strain A, the first attempted, eighteen tubes of medium were inoculated: four contained inactivated human serum and Locke's solution, 1:8, two of these with rice starch added and two without; six tubes contained inspissated horse serum (Loeffler's) with a cover fluid of serum and Locke's solution as described, three with rice starch and three without, and eight tubes contained Boeck's medium, with inactivated human serum in the cover fluid, four of these with rice starch added and four without. To one starch-free tube of each kind of medium used was added 0.3 cc. of sterile potato extract. This had no appreciable effect on the amebic growth and was used in growing one subculture only.

The first reading of cultures, forty-eight hours later, showed growth graded 1 and 2 on a basis of 1 to 4 (1 indicating slight growth and 4, large growth) in the first and simplest medium, without starch. There was no growth in the second group, in which the medium contained inspissated horse serum as a base. Growth graded 2 and 3 occurred in all of the eight tubes containing the egg slant as a base. From four of the best of the tubes showing positive growth, sixteen subcultures were

made at the end of the first forty-eight hours. Two days later, every one of these was negative. Examination of the original tubes, however, showed that three of those containing Boeck's medium with rice starch added, and, at that time, ninety-six hours old, still showed a growth graded 2. It was from these that the strain was continued, with the use, throughout, of the same medium as a standard, with an occasional modification of it for experimental purposes.

During the life of this strain, serums from the human being, the horse and the dog were used, both fresh and inactivated, without noticeable effect on the growth. On several early attempts to grow the ameba in a medium free from starch, it was found that the culture soon ran out and disappeared. It finally became possible to grow the ameba



A B

Fig. 1.—*A*, living, motile Endameba histolytica from culture (strain A) showing elongated shapes assumed by the organisms when moving across the field. *B*, motile forms of Endameba histolytica from culture (strain A) fixed and stained with iron-hematoxylin; × 300.

on a starch-free medium to which had been added 0.5 per cent defibrinated rabbit blood, according to the observations of Kofoid and Wagener.[24] The growth here, however, was far less than in the medium that contained starch. One attempt to grow *Endameba histolytica* on the simplified medium of Craig, that without the egg slant base, did not result in survival in any tube at the end of twenty-four hours.

Strain *A* grew luxuriantly on the Boeck and Drbohlav medium with the addition of rice starch (fig. 1). Growths in many of the tubes were graded as high as 3 and 4, particularly when the strain became older. It could be safely carried by inoculating only two or

three tubes each time a subculture was made. It seems reasonable to suppose that this particular strain might have been continued indefinitely. It was lost, however, at the end of seventy-two days, by accident, after it had passed through thirty generations or twenty-nine subcultures.

Strains D, E, F and G were inoculated into the medium previously used, the medium of Boeck and Drbohlav and starch, with no growth of D and poor growth and growth of short duration of the remainder.

In the cultivation of strain H, the modified medium of Boeck was used as described, except that to the overlying fluid in a portion of the tubes acriflavine was added in the proportion of 1 : 30,000. Later readings of these cultures showed growth graded 1 and 2 in the tubes without acriflavine, while in those with acriflavine the growth was graded 2 and 3. Subcultures from these, in the corresponding medium, continued to show growth in favor of the medium containing acriflavine; the growth was graded 4, 3, 3 and 2, respectively, in four tubes. Subcultures from these four tubes, without acriflavine, showed no growth in one tube and growth graded 1 in three tubes. The poorer cultures were dropped, and the strain was continued for a time with cultures in Boeck's medium and starch, to which acriflavine was added. Later attempts to continue the culture in mediums free from acriflavine showed that the importance of the latter is not great after a culture is established. Later, parallel cultures in mediums differing only in the content of acriflavine gave practically parallel growths.

Three attempts were made to grow *Endameba histolytica* of strain H on the simplified medium of Craig: one in the thirteenth generation of the strain, one in the twenty-seventh and one in the twenty-ninth. On the first attempt, Locke's solution and horse serum were used in the proportion of 7 : 1; in the second, Locke's solution and human serum, 7 : 1, and in the third, 0.85 per cent solution of sodium chloride and human serum in the same proportion. Readings of the first culture after twenty-four hours gave four amebas to a slide from one tube and none to a slide from a second. Twenty-four hours later, growth in both tubes was negative. The second attempt with Craig's medium gave grades of 1, 3 and 3 at the end of twenty-four hours, and of 2, 2 and 2 at the end of forty-eight hours. Growth in these tubes and in the tubes containing subcultures from them was negative twenty-four hours later. In this cultural attempt, it was found that a certain amount of the rice starch from the standard medium from the growth on which the cultures were made had been carried across to the Craig medium along with the transplanted amebas. This starch was seen microscopically at the time of the first examination, both within and outside the amebas. In the first subculture that was negative, starch was not visible. The third cultural attempt with the Craig medium gave but two amebas in a whole slide from one tube, one ameba in each

preparation from a second tube and negative results in preparations from the third tube. These cultures, as well as the subcultures from them, were negative twenty-four hours later.

In the twenty-fifth generation of strain H, one of the simplified mediums of Dobell was inoculated. This medium consisted of Ringer's solution, 8 parts, human serum, 1 part, and rice starch. This substrain grew well for three generations, or seven days; the growths at this time were graded 3 and 4. Surprisingly, on the next day, there were no organisms in any of the tubes. A second attempt, with the same simple medium in the thirtieth generation of this strain, did not show growth in twenty-four hours. A third attempt, in the thirty-second generation of strain H, gave a poor growth at the end of twenty-four hours, but twenty-four hours later there was no growth in these same tubes or in those containing the subcultures from them. With the use of the same medium, with the disodium hydrogen phosphate buffer added, inoculation in the thirty-eighth generation gave an amebic growth from fair to good for fifteen days, or six generations; then the strain was abandoned.

Strain H grew one hundred one days, or for forty-four generations, and was dropped when the need for it was passed. It never grew as profusely as strain A and was maintained with greater difficulty. It seems, however, that it might with care, have been maintained indefinitely.

Strain I grew poorly and for a short time only on Boeck's medium with starch added. On the same medium, to which was added acriflavine, strain J, from the stool contaminated with urine, gave a poor growth in the first generation only. In the same medium, strain K gave no growth. As has been indicated, this stool was cold at the time the inoculation was made.

Strain L was planted in three sets of mediums, one set containing Boeck's medium, with starch and acriflavine added; one containing the same, without acriflavine, and one containing inactivated human serum and Ringer's solution, without the underlying egg slant as recommended by Craig.[26] In all tubes growth was present at the end of the first forty-eight hours. In Craig's medium, however, growth was graded 2 as against that graded 2, 3 and 4 in the modified Boeck's medium. Twenty-four hours later, Craig's medium was negative for growth, both in the original tubes and in the tubes containing subcultures from them. Parallel subcultures on the egg base mediums with acriflavine modifying one set gave continuously from good to fair results for sixteen days, or eight generations, then the strain was abandoned. An attempt in the sixth generation to grow the established strain on the mixture of physiologic solution of sodium chloride and serum, the

medium of Craig, showed amebas at the end of forty-eight hours, but twenty-four hours later showed no growth in these tubes or in the tubes containing the first subcultures from them.

Although subcultures were made every forty-eight hours, on an average, survival in the same tube lasted as long as one hundred forty-four hours twice in strain A and once in strain H. Survival for one hundred twenty hours was reasonably frequent in all strains, and several times strains were saved by inoculation from tubes containing cultures of amebas ninety-six hours old. This is far from the experience of others in the field, whose cultures were maintained in the same tube for as long as twenty-four or more days, and who found it necessary to make subcultures at weekly intervals only. With my cultures, survival for the longer time could never be predicated, and for the sake of safety, an interval well within the maximum was chosen.

Early in the life of strain A, cysts were so common that many tubes contained a preponderance of these forms. As the strain grew older, cysts became less frequent and finally disappeared, to reappear in small numbers at later and irregular intervals. This is in conformity with the observations of Dobell and Laidlaw [23] on the effect of starch in the culture medium on production of cysts. None of my other strains produced more than a moderate number of cysts at any time, and all fell far short of the number of cysts produced by the first strain grown. Excystation was not observed, either in the fresh, living preparations or in the stained slides, but this particular activity of the culture cysts was not especially studied and might well have been overlooked although present (Dobell [34] and Yoshida [35]).

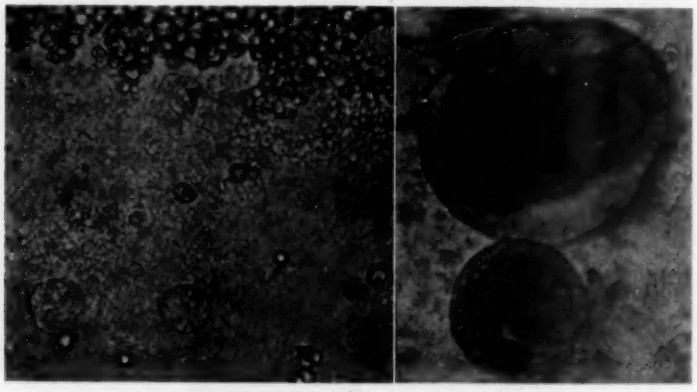
In unstained preparations of the cultivated amebas, the sudden cooling produced in preparing the slide caused temporary cessation of active motility. This was soon restored and lasted for half an hour or more in the same preparation, in spite of the concentration of the material due to the drying at the edges of the coverslip and of the temperature (that of the room) to which the amebas were exposed. Movement was usually typically progressive, with the amebas elongated in the direction of movement (fig. 1). Certain organisms, particularly the larger forms, remained in one situation and threw out clear pseudopodia in one or several directions. An occasional giant form was found to stretch out until the two resulting portions were united by a thread (fig. 2). In these cases, the widely separated halves were usually drawn together again. It happened, however, that this fine connecting thread

34. Dobell, Clifford: Further Observations and Experiments on the Cultivation of Endamoeba Histolytica from Cysts, Parasitology **19**:288, 1927.

35. Yoshida, Kazuyoshi: Reproduction in Vitro of Entamoeba Tetragena and Entamoeba Coli from Their Cysts, J. Exper. Med. **32**:357, 1920.

snapped while under observation, and the two resulting amebas went on their separate ways. This was observed three times. In one instance, the larger portion resulting from an unequal division of an ameba grown by culture, again divided in a similar manner. There thus took place the division of one large ameba into three amebas of about the average size. Dobell, in his extensive experience with cultivated amebas, never saw a living form divide, although he referred to York as having observed the process.

Especially conspicuous in the culture preparations was that form of débris-carrying ameba originally described as *Endameba sinensis* by Faust.[36] That portion of the ameba opposite the direction of movement had attached to it masses of bacteria that clung tenaciously and were not loosened by the relatively large and heavy starch granules among which



2 3

Fig. 2.—Living, motile Endameba histolytica from culture (strain A) showing one large, elongated ameba at the edge of a mass of starch granules. This ameba was later observed to divide. In the same field are two rounded, motile amebas of about average size.

Fig. 3.—Motile forms of Endameba histolytica from culture (strain A) stained with iron-hematoxylin. Differences in size are shown, whereas one only, the larger, shows an ectoplasmic margin, coarse nuclear rings and karyosomes; × 1250.

it flowed. Morphologically, the unstained amebas were similar to the forms described as occurring in the stools in cases of amebic dysentery. The nucleus was usually visible, but inconspicuously so. The size of organisms observed, however, was most variable; large and small forms were found side by side (fig. 3). The ectoplasm, too, varied in a similar manner; some amebas showed a relatively large amount and

36. Faust, E. C.: A New Type of Amoeba Parasitic in Man Observed in North China J. Parasitol. **9**:221, 1923.

some showed little if any (fig. 3). In preparations from mediums containing starch, the presence of starch granules within the amebas was conspicuous. In fact, some were so loaded with starch that it appeared to be with great difficulty that they dragged themselves around. At times, only careful observation made it possible to recognize that a large clump of starch granules was within an ameba. In the absence of starch from the medium from which the preparation was taken, bacteria were visible within the amebas, and under this condition the amebas presented the vacuolated, granular appearance usually ascribed to *Endameba coli.* These appearances in the fresh unstained preparations of amebas obtained in culture correspond to those previously observed by Boeck, Dobell, St. John and others.

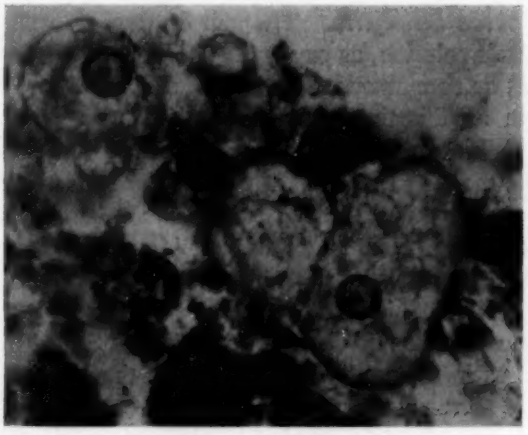


Fig. 4.—Endameba histolytica from an intestinal lesion of the kitten, stained with iron-hematoxylin, showing the lightly staining cytoplasm and the delicate nuclear rim, with its small, central karyosome. These are characteristics of the organism as found in lesions in tissue; × 1250.

When amebas obtained in culture were stained by the iron-hematoxylin method, the starch and bacteria ingested were accentuated in the fresh preparation, and the great variation in the size of the organisms was brought out more clearly, as well as the marked differences in the amount of ectoplasm. The most conspicuous disclosures of the stain, however, lay in the condition of the nucleus. Here was found a heavier, more granular nuclear ring of chromatin than usually is ascribed to *Endameba histolytica* (figs. 3 and 4). By this change, the nuclear ring in the cultivated organisms approached closely the appearance of the nuclear ring of *Endameba coli.* In the cultivated *Endameba histolytica,* this condition of the nucleus was not constant, however, and seemed to depend in part on the depth of staining. Although the karyosome

showed a similar unusual heaviness, it continued to be centrally placed. The occasional partial dispersion of the karyosome did not give me the impression that I was dealing with more than one species of ameba (Kofoid [37]). In the many stained preparations from all the strains grown, the giant form of York and Adams [38] was seen two or three times, although it fell far short of containing the thirty or forty nuclei described by them.

The table showing the complete protocol of these strains is omitted, owing to its voluminous nature.

CONCLUSIONS FROM STUDY I

In regard to culture mediums, conclusions are as follows:

The original medium of Boeck and Drbohlav is best for the cultivation of *Endameba histolytica.* The substitution of a sugar-free cover fluid for Locke's solution, as suggested by Drbohlav,[21] and the addition of rice starch, as recommended by Dobell and Laidlaw,[29] constitute the most valuable modifications of these basic mediums. Although the effect is not striking, the addition of acriflavine to the supernatant fluid portion of the medium for the first one or two transplants may help to establish the continuous growth of cultivated amebas.

Dobell's simplified medium, without solid constituents, gives variable results, indicating that older strains, modified by prelonged culture, might grow in it indefinitely.

The simple mediums of Craig do not give continued growth. Readings of cultures in the mediums suggest that the positive results might have been due to survival only.

In regard to the cultivation of *Endameba histolytica,* conclusions are as follows:

Cultivated *Endameba histolytica* usually retains its motility unimpaired. Amebas artificially grown show conspicuous variations in size. Under the cultural conditions of these experiments, formation of cysts is irregular and tends to appear early in the life of strains grown on mediums containing starch. Ingestion of bacteria and starch granules results in the appearance of increased vacuolation in the amebic cytoplasm. Variations in the amount of ectoplasm make its presence or absence of little value in the diagnosis of cultivated *Endameba histolytica.* Artificially grown *Endameba histolytica* shows increased coarseness of the chromatic material of the nucleus, which causes it to

37. Kofoid, C. A.: Councilmania Tenuis and C. Dissimilis, Intestinal Amebas of Man, Arch. Int. Med. **41**:558, 1928.

38. York, Warrington; and Adams, A. R. D.: Observations on Entamoeba Histolytica: I. Development of Cysts, Excystation, and Development of Excysted Amoeba in Vitro, Ann. Trop. Med. **20**:279, 1926.

approach, in appearance, the chromatin-rich nucleus of *Endameba coli*. The karyosome of the cultivated organism, however, continues to be centrally placed.

APPENDIX TO STUDY I

Cutler's Medium.—Cutler's mediums were prepared as follows:

1. One egg was shaken and to this 300 cc. of distilled water was added; the mixture was slowly brought to the boiling point on a water bath and boiled half an hour, with violent shaking throughout. The material was then placed in tubes, 5 cc. in each, and autoclaved. To each tube was added a few drops of blood just before inoculation.

2. Human blood clot to the amount of 500 cc. was boiled with 1,000 cc. of water for one hour. To the filtrate was added 0.5 per cent sodium chloride and 1 per cent peptone. This mixture was tubed and steamed for twenty minutes on three successive days. A few drops of blood were added just before use.

Yoshida's Medium.—Yoshida [11] tried several mediums, but only one giving "good" results was described: Ringer's solution, 4 parts, was added to horse serum, 1 part, and a few erythrocytes. The inoculated tubes were incubated at 22 and 27 C.

Boeck and Drbohlav's Mediums.—Boeck and Drbohlav's mediums were prepared as follows:

1. Locke's egg-serum (L. E. S.) was prepared as follows: Four eggs were washed, brushed with alcohol, then broken and added to 50 cc. of Locke's solution. The material was then tubed, slanted in an inspissator at 70 C. until solid, then autoclaved at 15 pounds (6.8 Kg.) pressure for twenty minutes. To the prepared slants was added a mixture of inactivated human blood serum and Locke's solution in the proportion of 1:8. This was added in an amount sufficient to cover the slant to a depth of about 1 cm. above the highest part.

2. Locke's egg-albumin (L. E. A.) was the same medium as Locke's egg-serum (L. E. S.), except that the cover fluid was a 1 per cent crystallized egg albumin in Locke's solution. Locke's solution, as used by Boeck and Drbohlav, was made as follows: Distilled water, 1,000 cc.; sodium chloride, 9 Gm.; calcium chloride, 0.2 Gm.; potassium chloride, 0.4 Gm.; sodium bicarbonate, 0.2 Gm., and dextrose, 2.5 Gm. The medium had a p_H of from 7.2 to 7.8 and served best when incubated at 37 C.

Drbohlav's Medium.—In Drbohlav's medium, "ovomucoid" solution composed of modified Ringer's solution was used as a cover fluid, to which was added a buffer, and the whole was adjusted by the addition of sodium hydroxide or potassium hydroxide to a p_H of 7.4. The buffered Ringer's solution was made as follows: Distilled water, 1,000 cc.; sodium chloride, 6 Gm.; potassium chloride, 0.1 Gm.; calcium chloride, 0.1 Gm.; sodium bicarbonate, 0.1 Gm., and monopotassium phosphate, 5 Gm. To this was added the white of one egg so carefully handled that the resulting mixture was used without filtering to sterilize. For the solid portion, one of the following three mediums was used, the best being the one containing starch: (1) Ringer's solution buffered as described (p_H 7.4), 1,000 cc., and agar, 14 Gm., autoclaved for thirty minutes, tubed and slanted; (2) same as 1, except that blood of any animal was added, and the mixture tubed, slanted and steamed at 100 C. for thirty minutes; and (3) same as 1, except that starch, 14 Gm. to the liter, was added. This was autoclaved thirty minutes, tubed and slanted. To each solid medium was added "ovomucoid" solution to cover the slant.

Dobell and Laidlaw's Medium.—In Dobell and Laidlaw's medium, for the solid medium already in use, inspissated horse serum slant (Loeffler's) was substituted and found satisfactory. For cover fluid, ovomucoid solution containing the whites of four eggs to 1,000 cc. of Ringer's solution (modified) was used; horse serum and Ringer's solution (modified) were used in the proportion of 8:1. Just before inoculation, a small amount of sterilized rice starch was added to all mediums used. The modified Ringer's solution was made as follows: Distilled water, 1,000 cc.; sodium chloride, 9 Gm.; potassium chloride, 0.2 Gm., and calcium chloride, 0.2 Gm. To sterilize the rice starch, a small amount of loose, dry starch (from 2 to 2.5 Gm.) was added to each of several test tubes. These were corked and heated to 180 C. dry heat for one hour.

Laidlaw, Dobell and Bishop's Medium.—The modified Ringer's solution of Dobell and Laidlaw was used, and a small amount of sterilized rice starch. Later, this was modified by adding, to the Ringer's solution for a buffer, 2 Gm. of disodium hydrogen phosphate.

Craig's and Craig and St. John's Mediums.—The mediums of Craig and of Craig and St. John are given in the body of the text.

Technic.—For the purpose of fixing amebic stools and amebic culture slides for staining, Schaudinn's fluid was used at room temperature only.

In experiments with Heidenheim's technic for staining with iron-hematoxylin, it was found that immersion of the slide in 0.5 per cent aqueous solution of hematoxylin for half an hour was adequate, when the slide had been mordanted for half an hour in an aqueous solution of iron-alum.

2. INOCULATION OF ANIMALS

DATA FROM THE LITERATURE

The first recorded successful attempt to infect an animal with amebic dysentery by inoculating it with ameba-containing material was made by Lösch [39] (1875). At first, he fed three dogs and gave them rectal injections of the freshly passed stool of a patient who had active amebic dysentery. Infection did not result. In an attempt to prepare the bowel for the ready invasion of the injected amebas, he gave a fourth dog a preliminary dose of croton oil. When the immediate effects of this had subsided, he introduced into the colon of this animal, by rectal injection, on each of three successive days, a freshly passed ameba-containing dysenteric stool. Although the dog remained clinically well, on the eighth day, examination of the stool disclosed the presence of motile amebas resembling those injected. Eighteen days after the last injection, the dog was killed, and motile amebas indistinguishable from those contained in the injected human stool were found in the ulcerative lesions of the colon.

Uplavici [40] obtained only two positive results in inoculations of seventeen dogs. The use of cats, however, gave four infections in the

39. Lösch, F.: Massenhafte Entwickelung von Amöben in Dickdarm, Virchows Arch. f. path. Anat. **65**:196, 1875.

40. Uplavici, O. (Hlava, O.), quoted by Kartulis (footnote 41).

six animals used. Negative results were reported from the inoculation of eight young dogs, two chickens and six guinea-pigs. The methods used by Uplavici were not detailed, but the results with the cat indicated that it is rather highly susceptible to infection with *Endameba histolytica*.

Kartulis [41] reported on the results in three cats of rectal injection of human dysenteric stool containing large numbers of motile amebas. One of the three cats had an infected stool in two days and died of dysentery in fourteen days. Postmortem examination of the cat did not disclose definite ulceration, but widespread erosion of the colon, with punctate hemorrhages. The other two cats remained normal. Some doubt is cast on the validity of the observations because of the results obtained by the same author with cultures of these amebas. He inoculated the amebas of dysentery into a simple straw infusion, and claimed to have found them, later, growing among the numerous bacteria on the surface of this fluid in a weblike pellicle. That the organisms obtained here were free-living forms is evidenced by the nature of the culture medium, their situation on the surface of it and their ability to excyst after a lapse of time as great as four months. With this material for injection, Kartulis reported the production of amebic dysentery in cats. He further reported positive intestinal lesions in one of three cats each of which was given a rectal injection of a pure (bacteria-free?) culture of the amebas of dysentery in the third cultural generation. Feeding experiments with cultivated cysts gave negative results. In spite of the great probability that Kartulis did not produce amebic dysentery in animals by the injection of his cultivated amebas, he introduced into his experiments the control element of first examining the stool of the animal to be used, in order that native parasitic amebas found in it should not add an element of confusion to the result. He gave each animal a preliminary dose of morphine, and in two instances sewed the anus of the inoculated animal with catgut to prevent the early expulsion of the injected material. These sutures were removed at the end of two and three days.

Kovacs [42] continued the use of cats in the experimental production of amebic dysentery. He introduced material from the dysenteric stool of two patients into the bowels of cats, both by enema and by injection of the infectious material directly into the small bowel by means of a needle. In some instances, the anus of the animal was closed by suture for twenty-four hours following the inoculation. He found the ameba-containing stool of patients to be infectious for cats only during the

41. Kartulis, S.: Einiges über die Pathogenese der Dysenterieamöben, Centralbl. f. Bakteriol. **9**:365, 1891.

42. Kovacs, F.: Beobachtungen und Versuche über die sogenannte Amoebendysenterie, Ztschr. f. Heilk. **13**:509, 1892.

period of active dysenteric symptoms. For diagnosis, fecal material of the suspected animal was obtained by the introduction of a glass rod into the rectum.

This work was later extended and continued by a long line of investigators (Quincke and Roos,[43] Kruse and Pasquale,[44] Marchoux,[45] Craig,[46] Werner,[47] Dale and Dobell,[48] Sellards and Leiva [49] and others), all confirming, in part, the previous work of pioneers in the field, amplifying and modifying it.

Kruse and Pasquale [44] reported on the rectal injection into forty kittens of stools from clinical cases of amebic dysentery. They were the first to inoculate kittens successfully by rectal injection of pus from an amebic abscess of the liver. Marchoux,[45] and Strong and Musgrave [50] also succeeded in producing dysentery by rectal inoculation of cats with material obtained from tropical abscess of the liver. The latter produced intestinal lesions in both of two cats used, one showing definite lesions of the colon in two days, the other presenting more advanced lesions in five days. The pus from the amebic abscess of the liver used was found to be free from bacteria, both on direct examination and on culture. Later attempts (Werner,[47] Wenyon [51]) to inoculate cats by the rectal injection of ameba-containing pus from amebic abscesses of the liver failed.

Quincke and Roos [43] mentioned the kitten rather than the cat as their experimental animal, and were the first to recognize and describe

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43. Quincke, H., and Roos, E.: Ueber Amöben-Enteritis, Berl. klin. Wchnschr. **30**:1089, 1893.

44. Kruse, Walther; and Pasquale, Alessandro: Eine Expedition nach Egypten zum Studium der Dysenterie und des Leberabscesses, Deutsche med. Wchnschr. **354**:378, 1893; Untersuchungen über Dysenterie und Leberabscess, Ztschr. f. Hyg. u. Infectionskrankh. **16**:1, 1894.

45. Marchoux, M.: Note sur la dysenterie des pays chauds, Compt. rend. Soc. de biol. **51**: 870, 1899.

46. Craig, C. F.: Observations Upon Amebas Infecting the Human Intestine, with a Description of Two Species, Entamoeba Coli and Entamoeba Dysenteriae, Am. Med. **9**:854, 897 and 936, 1905.

47. Werner, Heinrich: Studien über pathogene Amöben, Arch. f. Schiffs-u. Tropen-Hyg. **12**:1, 1908.

48. Dale, H. H., and Dobell, Clifford: Experiments on the Therapeutics of Amoebic Dysentery, J. Pharmacol. & Exper. Therap. **10**:399, 1917.

49. Sellards, A. W., and Leiva, L.: Investigation Concerning the Treatment of Amoebic Dysentery, Philippine J. Sc. **22**:1, 1923; The Effect of Stasis in the Development of Amoebic Dysentery in the Cat, ibid. **22**:39, 1923.

50. Strong, R. P., and Musgrave, W. E.: Preliminary Note Regarding the Etiology of the Dysenteries of Manila, Annual Report of the Surgeon General, U. S. Army, 1900, p. 251.

51. Wenyon, C. M.: Protozoology, New York, William Wood & Company, 1926, pp. 225-234.

the encysted form of *Endameba histolytica.* After feeding the cyst-containing stool from human beings to four kittens from two to nine days after the stool was passed, they demonstrated the development of amebic intestinal ulceration in two animals. They found the feeding of stools containing only the motile forms of *Endameba histolytica* to be without effect. Schaudinn [2] fed two cats the dried stools of a patient who had amebic dysentery. One became infested in three days and showed, at death, lesions of amebic dysentery. The second likewise became ill, but recovered in four weeks. A third cat was fed the infected stool of an infected animal without effect. Four weeks later, this cat was fed a dried stool from a dysenteric patient, and intestinal amebiasis developed. Schaudinn first clearly recognized and described two species of parasitic amebas in man, the harmless *Endameba coli* and the pathogenic *Endomeba histolytica.* He recognized the cysts of the former, but believed that *Endameba histolytica* produced only a resistant spore.

In marked contrast with the observations of Quincke and Roos and Schaudinn, Craig [46] reported that when half-grown kittens were fed with stools from dysenteric patients, 65 per cent of the animals showed amebic dysentery as compared with 50 per cent of animals that were given rectal injections of the same material. The results of his feeding experiments are unexplained, unless the cysts of *Endameba histolytica* were actually present but unrecognized, since, at that time, following the teaching of Schaudinn, he doubted that *Endameba histolytica* encysts. He recognized the cysts of *Endameba coli,* however, and feeding experiments conducted with them gave negative results. Werner,[47] on the other hand, was unable to infect animals by feeding them with the motile forms of *Endameba histolytica* or of *Endameba tetragena* (organisms which he felt to be identical) either from human beings or from cats recently dead of amebic dysentery. Huber [52] found the feeding of trophozoïtes to be innocuous. Schimura,[53] who recognized the cystic forms of *Endameba histolytica,* found the incidence of infections resulting from feeding experiments conducted with them to be 50 per cent as contrasted with an incidence of 90 per cent in cases in which the motile forms were injected rectally.

In the feeding experiments for the production of infection with endamebas, the classic work of Walker and Sellards [54] should occupy

52. Huber: Untersuchungen über Amöbendysenterie, Ztschr. f. klin. Med. **67:** 262, 1909.

53. Schimura, Sohei: A New Non-Pathogenic Tetragenous Ameba, J. Exper. Med. **28:**415, 1918.

54. Walker, E. L., and Sellards, A. W.: Experimental Entamoebic Dysentery, Philippine J. Sc. **8:**253, 1913.

first place in importance if not in point of time, because of the significance of the results obtained. Their work, instead of being done on the usual experimental animal, was done on men, volunteers from among the native prisoners of a Philippine prison. Of the sixty men used, twenty were fed free-living amebas and the cysts of free-living amebas, without result. Twenty were fed cysts and motile forms of *Endameba coli,* and the organism appeared in the stools of seventeen in from two to eleven days. Neither of these groups presented any intestinal symptoms of infection. Finally, twenty men were fed the cysts and motile forms of *Endameba histolytica.* Of these, seventeen had stools containing *Endameba histolytica* after the first feeding, whereas one required three feedings to establish parasitization and two remained uninfected. In four of the group in whose stools *Endameba histolytica* was found, amebic dysentery developed, but they recovered from it. It was clearly shown that *Endameba coli* and *Endameba histolytica* may become established as parasites in the colon of man, through his ingestion of the cyst forms. Feedings of the motile forms of these organisms did not show conclusively, however, that they might not also be infectious following ingestion. Strangely enough, attempts to inoculate animals with *Endameba histolytica,* both by feeding of cysts and by rectal injection of motile forms, did not result in infection in seven monkeys, two cats, six kittens and one pig.

Marchoux [45] was the first to carry amebic dysentery from animal to animal by inoculating one animal with the infected stool of another. He spoke of the ease with which the cat is thus infected, and succeeded in carrying one strain of *Endameba histolytica* through nineteen animal passages. Wenyon,[45] using stools of human beings containing cysts and motile forms of *Endameba histolytica* to infect the first animal, carried one strain through four subinoculations in cats. Similar propagation of animal dysentery, by inoculation from animal to animal, was carried out by Darling,[55] Dale and Dobell, Mayer,[56] Sellers and Leiva,[57] Werner, Wagener [58] and others. Attempting to insure the survival of animal strains of *Endameba histolytica,* Sellards and Baetjer [59] introduced the motile forms of this organism into the cecum by means of

55. Darling, S. T.: The Identification of the Pathogenic Entamoeba of Panama, Ann. Trop. Med. **7**:321, 1913.

56. Mayer, Martin: Klinische, morphologische und experimentelle Beobachtungen bei Amöbenerkrankungen, Arch. f. Schiffs-u. Tropen-Hyg. **23**:172, 1919.

57. Sellards and Leiva (footnote 49, first reference).

58. Wagener, Edna H.: A Precipitin Test in Experimental Amoebic Dysentery in Cats, Univ. Calif. Pub. Zool. **26**:15, 1924.

59. Sellards, A. W., and Baetjer, W. A.: The Propagation of Amoebic Dysentery in Animals and the Recognition and Reproduction in Animals of Atypical Forms of the Disease, Am. J. Trop. Dis. **2**:231, 1914.

a needle. In this way, they carried one strain through eleven generations of cats with positive infections resulting in about 100 per cent as contrasted with positive infections in 65 per cent of animals that received injections by rectum. Sellards and Leiva [60] went a step further in the operative inoculation of animals. In addition to the injection of the infective material into the cecum by means of a syringe and needle, they ligated the bowel at various levels below this point and reported the production of dysenteric lesions above the ligature in all of three kittens used. They emphasized the value of stasis for the production of infection in the animals that were given injections. Sellards and Theiler [61] carried out the same operative procedure with the introduction of the cysts of *Endameba histolytica,* and demonstrated the ability of the parasite to excyst within the colon of the experimental animal.

Early experimental work tending to show that *Endameba histolytica* and *Endameba tetragena* are probably environmental modifications of the same organism was that of Werner.[47] In the attempted propagation of animal strains by inoculating one animal with the positive stool of another, he succeeded in carrying three strains of *Endameba tetragena* through five, three and one animal passages and carried one strain of *Endameba histolytica* through six.

Cutler [8] was the first to succeed with cultures of *Endameba histolytica* for inoculation into animals. Although some have doubted that Cutler was able to grow *Endameba histolytica,* it is now known that he may have grown them and probably did (see study 1). His cysts in cultures given by mouth infected five of the six kittens used, whereas rectal injection of the motile forms, from culture, infected the only kittens used. The cultivated organisms of Boeck and Drbohlav [10] and of Drbohlav [62] were found to be infectious. With them, the rectal injection of motile forms of *Endameba histolytica* from culture produced amebic dysentery in 65 per cent of the sixteen kittens used. It was their observation that length of life under cultivation did not modify infectivity. The recent work of Kessel [63] confirmed the observations of previous workers with reference to the infectiousness of cultures of *Endameba histolytica.*

Dobell and Laidlaw [23] reported that the addition of rice starch to the culture medium in which *Endameba histolytica* is grown apparently destroyed their infectivity for cats. They gave seven cats rectal injec-

60. Sellards and Leiva (footnote 49, second reference).

61. Sellards, A. W., and Theiler, M.: Investigations Concerning Amoebic Dysentery, Am. J. Trop. Med. **4**:309, 1924.

62. Drbohlav (footnote 18, third reference).

63. Kessel, J. F.: Amoebiasis in Kittens Infected with the Dysentery Amoeba from Acute and Carrier Human Cases and with the Tetranucleate Amoeba of the Monkey and the Pig, Am. J. Hyg. **8**:311, 1928.

tions of the thirteenth subculture of *Endameba histolytica* grown in a starch-containing medium, with negative results in all. It was their belief, based on the results obtained with one inoculated animal, that this infectivity, once lost, could not be restored by cultivation on mediums free from starch. That the conclusions of Dobell and Laidlaw are not strictly justifiable was shown by Rees,[64] who introduced the motile forms of *Endameba histolytica* grown on starch-containing medium into the bowels of experimental animals in which the colon had been occluded by ligature near the rectum. In this way, he produced infection with *Endameba histolytica* in about 50 per cent of the animals used.

Abscess of the Liver in Experimental Animals.—The development of abscess of the liver in artificially infected animals was first described by Marchoux [45] in the case of a cat that died of amebic dysentery. Similar observations were reported later by Craig,[46] Werner, Huber, Wenyon,[5] Dale and Dobell, Mayer and others. Harris [65] observed abscesses of the liver in two of three very young dogs inoculated rectally with stool from a dysenteric patient.

Special Methods.—Special methods of handling the experimental animals have included the administration of a preliminary cathartic (Lösch, Rees [66]), preliminary starvation (Rees [66]) and the administration of an anesthetic for the injection of infectious material, both for operative introduction and for simple injection through a catheter by rectum (Sellards and Leiva,[49] Sellards and Theiler, Rees,[67] Sanders [68]). Attempts to produce stasis after inoculation have varied from the simple expedient of holding the cat head down for a few minutes after the injection (Dale and Dobell), through temporary plugging of the anus (Boeck and Drbohlav [10]), suture of the anus (Kartulis,[41] Kovacs [42]) and ligation of the bowel below the site of operative injection (Sellards and Leiva,[60] Sellards and Theiler,[61] Rees [69]). In only one case was stool from a dysenteric patient injected directly into the liver of an animal (Werner [47]). This was done in a guinea-pig, with negative results.

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64. Rees, C. W.: Experimental Amoebiasis in Kittens, J. Parasitol. **14**:125, 1927; The Infectivity and Pathogenicity of a Starch-Fed Strain of Endamoeba Histolytica, ibid. **15**:131, 1928.

65. Harris, H. F.: Experimentell bei Hunden erzeugte Dysenterie, Virchows Arch. f. path. Anat. **166**:67, 1901.

66. Rees (footnote 64, first reference).

67. Rees, C. W.: Pathogenesis of Intestinal Amebiasis in Kittens, Arch. Path. **7**:1, 1929.

68. Sanders, E. P.: Changes in the Blood Cells of Kittens Resulting from Infections with Endamoeba Histolytica, Am. J. Hyg. **8**:963, 1928.

69. Rees (footnote 64, second reference; footnote 67).

Animal to Animal Inoculation.—Infection carried from animal to animal by rectal inoculation with the stool of the infected cat resulted in the widest possible variations both in the incidence of "takes" and in the number of animals through which the different strains of *Endameba histolytica* were carried. Werner maintained 3 strains of *Endameba tetragena* for 5, 3 and 1 passage, respectively, and 1 strain of *Endameba histolytica* for 6 passages. Wenyon [5] carried a strain obtained from the stool of a human being through 4 passages. By operative inoculations, Baetjer and Sellards [70] carried 2 strains of *Endameba histolytica* from men for 11 generations. Wagener and Thomson [71] carried *Endameba histolytica* from the stool of the human being through 6 passages by the use of 23 cats. In marked contrast with these modest results are the reports of Marchoux, who carried 1 strain through 19 passages; of Mayer, who carried 3 strains through 32, 39 and 49 passages, respectively, with positive results in 86.5 per cent of 126 kittens used, and of Dale and Dobell, who carried 1 strain of *Endameba histolytica* through 43 animal passages in 120 kittens. In the work of the latter, amebic infection failed to develop in only 12 of 139 kittens. The high proportion of infections obtained by Dale and Dobell may be due, in part, to the fact that their material for inoculation was obtained by washing the opened bowel of a recently killed dysenteric animal with 0.9 per cent solution of sodium chloride and adding the scraping of the mucosa to obtain a rich suspension of *Endameba histolytica.*

Incubation.—The period of incubation, as determined for the commonly used cat, varied from one day (Dale and Dobell, Wagener and Thomson) to twenty (Baetjer and Sellards [70]). This varied definitely with the age of the animal (Sellards and Leiva [57]), and was shorter in those which were younger. It varied, also, with the nature of the inoculation, being longer in animals inoculated by the feeding of cysts (Dale and Dobell). In the attempt to fix more accurately the time of onset of amebic infection in the experimental animal, Sellards and Leiva [57] did not rest the diagnosis on the examination of stools passed spontaneously, but determined the onset of infection by the examination of stools obtained by means of an enema. Kovacs accomplished the same end by the use of a glass rod inserted into the rectum of the animal that had been given the injection.

Modification of Virulence of Endameba Histolytica.—Widely varying opinions are held concerning the effects of passage through animals

70. Baetjer, W. A., and Sellards, A. W.: Continuous Propagation of Amoebic Dysentery in Animals, Bull. Johns Hopkins Hosp. **25**:165, 1914.

71. Wagener, Edna H., and Thomson, Margaret D.: Experimental Amoebiasis in Cats from Acute and Chronic Human Cases, Univ. Calif. Pub. Zool. **26**:267, 1924.

and of artificial cultivation on the virulence of *Endameba histolytica.* Respecting the effects of passage through animals, it is believed, on the one hand, that this increases the infectivity of the protozoon (Baetjer and Sellards,[70] Meyer, Sellards and Leiva [57]), and on the other hand, that passage decreases its infectivity (Werner, Darling [55]). Darling ascribed the loss of virulence to the gradual reduction in size of *Endameba histolytica* by passage through animals, until it finally reaches a uninucleated cyst stage in the fourth or fifth subpassage, after which it ceases to be infectious. Wagener expressed the belief that virulence is unaffected. Cultural modification of *Endameba histolytica* was seen by Sanders, who observed loss in the infectivity of *Endameba histolytica* grown artificially. Boeck and Drbohlav [10] did not find a change after prolonged cultivation, whereas Dobell and Laidlaw found complete, unrestorable loss of virulence in *Endameba histolytica* grown on starch-containing mediums.

Wagener and Thomson alone found the resistance of the experimental animal to infection with *Endameba histolytica* to be increased by previous attempts at inoculation.

Choice of Experimental Animal.—In attempted inoculations of animals with *Endameba histolytica,* the dog and the cat usually have been used. Darling [72] described spontaneous dysentery in the dog due to *Endameba histolytica,* but usually the dog has been somewhat refractory to infection with this organism (Lösch, Uplavici). The cat, and particularly the young kitten, became the animal of choice. This animal is easy to procure and is highly susceptible to infection with *Endameba histolytica* and presents, when infected, ulcerative intestinal lesions closely resembling those of man. The guinea-pig, although frequently used (Uplavici, Werner, Baetjer and Sellards,[73] Chatton,[74] Wagener and Thomson), has proved refractory; when infected, this animal does not present symptoms of dysentery, and shows a peculiar tumor-like lesion of the involved bowel rather than the usual ulceration (Baetjer and Sellards,[73] Chatton [74]). Only two have reported the development of amebic lesions in the inoculated rabbit (Huber, Thomson [75]). This animal remained free from intestinal symptoms and the intestinal lesions

72. Darling, S. T.: Entamoebic Dysentery in the Dog, Proc. M. A. Isthmian Canal Zone **6**:60, 1913.

73. Baetjer, W. A., and Sellards, A. W.: The Behavior of Amoebic Dysentery in Lower Animals and Its Bearing upon the Interpretation of the Clinical Symptoms of the Disease in Man, Bull. Johns Hopkins Hosp. **25**:237, 1914.

74. Chatton, E.: Réalisation expérimentale chez le cobaye de l'amebiase intestinale à'Entamoeba dysenteriae, Bull. Soc. Path. exot. **10**:794, 1917; L'amebiase intestinale expérimentale du cobaye à Entamoeba dysenteriae, Arch. Inst. Pasteur de Tunis **10**:138, 1918.

75. Thomson, Margaret D.: Experimental Amoebiasis in the Rabbit, Univ. Calif. Pub. Zool. **29**:9, 1926.

that developed were far from comparable with those of the human being. The monkey has been used occasionally (Walker and Sellards, Dale and Dobell, Kessel [63]), but is subject to the development of spontaneous dysentery and has within its intestinal tract parasitic amebas that are indistinguishable from those of man and that are believed (Dobell and Laidlaw) to be identical with them. The rat has been used in the experimental production of amebic dysentery by many (Werner,[47] Wagener and Thomson, Brug,[76] Lynch,[77] Kessel,[78] Chiang [79]). The work of Lynch [77] and of Chiang tended to show that the monkey may be a carrier of *Endameba histolytica;* at least, with the mouse (Kessel [80]), it frequently harbors confusing parasitic intestinal amebas. Less frequently used animals are the chicken (Uplavici) and the pig (Walker and Sellards, Wagener and Thomson, Kessel [63]).

In the literature dealing with animals for experimental production of amebic dysentery, the terms "cat" and "kitten" have been loosely used, the general term "cat" often including the kitten even when this is not expressly indicated.

The domestic cat, as found throughout the world, varies widely in appearance and actions and may present enough differences in resistance to infection with *Endameba histolytica* to explain, in part, the varying results obtained by investigators working with the native cats of widely separated countries. So far, no investigator has named the species of cat used by him in his experimental work. Chatton [81] alone remarked on the special resistance of the native cats of south Tunis to infection with *Endameba histolytica.* Until it is shown that the domestic cat, wherever found, offers approximately the same resistance to experimental amebic dysentery, the results of experiments in inoculation conducted with *Felis chaus* of China or of *Felis manual* of Egypt should not be compared uncritically with those obtained with the domestic cat of America (*Felis domestica*).

In my selection of the animal best suited for the experimental work to be undertaken, everything pointed toward the kitten. Although for all the commoner laboratory animals native parasitic intestinal amebas

76. Brug, S. L., quoted by Wenyon (footnote 5).

77. Lynch, K. M.: The Rat a Carrier of a Dysenteric Amoeba, J. A. M. A. **65**:2232, 1915.

78. Kessel, J. F.: Methods of Obtaining Amoeba-Free Rats for Experimental Infection with Intestinal Amoeba, Univ. Calif. Pub. Zool. **20**:401, 1923; Experimental Infection of Rats and Mice with the Common Intestinal Amoebae of Man, ibid. **20**:409, 1923; The Distinguishing Characteristics of the Parasitic Amoebae of Culture of Rats and Mice, ibid. **20**:490, 1924; footnote 63.

79. Chiang, S. F.: Study of Parasitic Amoebae by Experimental Cross Infection of Laboratory Animals, Nat. M. J. China **11**:440, 1924-1925.

80. Kessel (footnote 78, second and third references).

81. Chatton (footnote 74, first reference).

have been described (Hegner and Taliaferro,[82] Craig,[83] Wenyon,[51] Knowles [84]), certain authors excluded the cat from the list of animals enumerated (Craig,[83] Hegner and Taliaferro, Wenyon [51]). Among 150 kittens obtained from the streets of Peiping, Kessel [63] found 3 ill with amebic dysentery. Wenyon [51] was of the opinion that the only ameba of the cat is the true *Endameba histolytica*. This comparative freedom of the cat from native amebiasis, the availability of the animal, the ease with which it is handled, its susceptibility to infection with *Endameba histolytica* and, finally, the character of the intestinal amebic lesions, determined my choice.

TABLE 3.—*Data Concerning Cases Which Furnished Material for Inoculation of Animals; Results of Inoculations*

Case	Age, Yr.	Sex	Where Contracted	Duration of Symptoms	Stool According to History	Forms Used	Animals Used	Amebiasis in Kittens
9	38	F	Texas	No definite number of days	Formed	Motile forms and cysts	4	Absent
10	25	M	Mexico	3 months	Formed	Cysts	2	Absent
11	33	M	Texas	2 or 3 years	Formed	Motile forms	3	Absent
12	55	M	Porto Rico	No history of dysentery	Formed	Motile forms	2	Absent
13	22	M	Illinois	1 year	8 to 9 each day, with blood	Motile forms	4	Absent
14	30	M	Texas	1 year	8 to 20 each day; occasional blood	Motile forms	4	Present in one
6	22	F	Minnesota	2 years	Early morning looseness	Motile forms	1	Absent
15	48	F	India	2 years	Formed	Motile forms	3	Absent
5	48	F	China	24 years	5 to 12 watery stools each day	Motile forms	2	Present in one
8	27	M	Illinois	2 years	5 to 14 each day; with mucus and blood; no salts	Motile forms	4	Present in four

MATERIAL INOCULATED AND METHODS USED

The cultivated amebas used for inoculation in animals were the vigorous strain A and the longer-lived but less vigorous strain H, previously described. Both were grown on Locke's egg-serum medium of Boeck and Drbohlav, to which was added rice starch as recommended by Dobell. Acriflavine in the proportion of 1:30,000 was added to the medium of strain H for a portion of its life.

Stools containing *Endameba histolytica* were obtained from ten patients who during the course of this experimental work (table 3) presented themselves at the Mayo Clinic for diagnosis and treatment. Of these patients, only four had at

82. Hegner, R. W., and Taliaferro, W. H.: Human Protozoology (Monograph), New York, The Macmillan Company, 1924, pp. 47-90.

83. Craig, C. F.: A Manual of the Parasitic Protozoa of Man, Philadelphia, J. B. Lippincott Company, 1926, p. 37.

84. Knowles, Robert: An Introduction to Medical Protozoology, with Chapters on the Spirochaetes and on Laboratory Methods, Calcutta, Thacker, Spink & Company, 1928, pp. 39-78.

the time active intestinal symptoms. Of the remaining six, two did not give a history of previous dysentery, while all but the four mentioned did not have, or had only very mild, intestinal symptoms.

The infected stools of kittens that had been given injections were also used.

For the purpose of introducing the infective material from either a culture or a stool, a 5 cc. glass Luer syringe was fitted with a rubber catheter tip so cut as to give a usable length of about 10 cm. (Wenyon).[5] This tip was flexible enough to follow the anatomic course of the bowel and yet stiff enough to overcome the ordinary resistance to its introduction. This catheter-tipped syringe was also used for the introduction of material into the stomach by way of the mouth in the "feeding" experiments.

Cultural material was always fluid enough to require no dilution for use with the syringe. Usually, typical dysenteric stool, free from particles of feces, could also be used undiluted. If the character of the stool was such that it could not be introduced by means of the syringe, it was diluted with warm physiologic solution of sodium chloride or tap water, was greatly broken up and was filtered through one layer of coarse gauze. When possible, feces were injected undiluted and immediately after being obtained.

In the selection of cultivated amebas for inoculation in animals, only those in tubes that showed good growth of live amebas were used. They were kept at incubator temperature throughout. Immediately before the amebas were to be used, the upper two thirds of the fluid portion of the medium in the selected tubes was siphoned away, the remaining fluid was agitated to stir up the ameba-containing starch sediment, and a heavy mixture of amebas and starch granules was thus obtained with a small amount of fluid. Of this mixture, from 3 to 4 cc. was used for each animal and never more than 5 cc.

It was necessary to transport the material from patients' stools some distance before it could be used experimentally. There was thus an appreciable lapse of time between the reception of the stool and its use. During this time, an effort was made to protect it against cooling. In one instance, a stool containing the motile form of *Endameba histolytica*, that had been kept in the incubator for three hours before use, produced amebic dysentery in all four inoculated half-grown kittens.

Special care was not used to protect cyst-containing material from culture or man against cooling.

For the simple injection of material by bowel or stomach, no preparation of the animal was attempted. For rectal injection, the catheter was inserted as far as it could be easily and safely passed, the material was slowly injected, and the animal was held head down for a variable time, up to as long as ten minutes, as suggested by Dale and Dobell. Occasionally, moderate attempts were made to produce early postinjection stasis by the simple expedient of Boeck and Drbohlav[10] of plugging the anus with cotton and sealing it over with collodion. This was hard to apply, remained a most uncertain length of time, and fell far short of producing the degree of stasis that resulted from the use of a ligature or suture. Because it was difficult to determine the value of this procedure, it was used only with some of the earlier inoculations.

Animals were prepared for operative inoculation by the withholding of food for twenty-four hours. The abdomen was opened under anesthesia; the injection was made into the cecum by means of a small needle, and in some instances the colon was ligated in its lower portion by means of nonabsorbable material. In all operative inoculations, cultural material only was used; this necessitated only a small needle and no particular care of the puncture wound in the bowel. Fol-

lowing the operation, the animals were offered water and milk, but forced feeding was not attempted. Early postoperative recovery was remarkable, and it was impossible to distinguish, by the actions of the animals, between those with ligated colons and those without.

For the diagnosis of dysentery in the inoculated kittens, the appearance of diarrhea alone could not be relied on. It was necessary to find *Endameba histolytica* in the stool of the cat. For this purpose, the suspected animal was given a rectal injection of a small amount of warm physiologic solution of sodium chloride by means of a syringe and catheter arrangement similar to that used for inoculation (Sellards and Leiva [57]). Without removal of the tip of the catheter from the bowel, manipulation resulted in drawing into the syringe some of the injected fluid stained with feces, blood and mucus, in some part of which the motile forms of *Endameba histolytica* were found, if the cat was infected. The estimation of the period of incubation was, in all cases, based on the time of the appearance of *Endameba histolytica* in the stools and not on the time of onset of intestinal symptoms. Occasionally, the time of incubation might have been found to be shorter by an earlier examination of the stool. Finally, all positive infections here reported were later confirmed by postmortem examinations.

Since the study of the lesions in the intestine of the infected dysenteric animal was the primary object of the experimental work, no animal was killed to procure material for animal to animal inoculation. Infected stool was obtained by means of the syringe and catheter in a manner similar to that exercised in obtaining stool for examination. This resulted often in a smaller quantity of material than was desired, and may explain, in part, my inability to carry the infections of the animals through a long series. It made possible, on the other hand, the use of the same animal as a source of supply over a period of one or more days, and so far as can be determined, left the intestinal lesions of the donor cat unmodified for later study.

From an examination of tables 4 to 7 inclusive, in which the details of the inoculations are given, it will be seen that some of the animals were used as many as four and five times; that an animal marked "negative" in one may appear as "positive" in another; that in some instances the same animal was used repeatedly at such short intervals that the estimate of the incubation period indicated is of no value, since the infection may have resulted from an injection other than the last. A study of the dates indicating the order of inoculation will show that the animal that showed negative results one or more times later showed positive results. Concerning the infecting injection, it can be said only that the so-called negative animal was considered to be negative if it seemed well and if examination of its stool gave negative results. The object throughout was the production of lesions of amebic dysentery, and not a critical study of the susceptibility of the cat to infection with *Endameba histolytica*.

RESULTS

Inoculation of Animals with Cultures of Endameba Histolytica.—Although Dobell and Laidlaw stated that the artificial cultivation of *Endameba histolytica* in mediums containing rice starch apparently destroyed their infectivity for animals, the first of my experiments in inoculation was undertaken with a rich culture of *Endameba histolytica*, strain A, previously described, grown in the medium of Boeck and Drbohlav, Locke's egg-albumin, to which rice starch had been added.

The first kittens available for this work were young, some as young as fourteen days, and the mortality among them was high owing to causes other than the experimental manipulations. With cultures of strain A, thirteen inoculations were made in five animals; kitten 2 was used three

TABLE 4.—*Data Concerning Inoculation of Animals with Cultures of Endameba Histolytica*

Kitten	Strain	Cysts Given by Mouth	Trophozoites Given by Rectum	Amebic Lesions	Length of Life After Last Inoculation, Days	Manner of Death	Comment
1	A	..	+	—	..	.....	
1	A	..	+	—	7	Died	
2	A	..	+	—	..	.....	
2	A	..	+	—	..	.....	
2	A	..	+	—	7	Died	
3	A	..	+	—	..	.....	
3	A	..	+	—	..	.....	
3	A	..	+	—	..	.....	
3	A	..	+	—	..	.....	
3	A	..	+	—	141	Died	
4	A	+	..	—	..	.....	
4	A	..	+	—	1	Died	
5	A	+	..	—	1	Died	
6	H	..	+	—	..	.....	
6	H	..	+	—	..	.....	
6	H	..	+	—	..	.....	
6	H	..	+	—	..	.....	
6	H	..	+	—	4	Killed	Stool positive for amebas
7	H	..	+	—	..	.....	Operative inoculation; rectum tied
7	H	..	+	—	..	.....	
7	H	..	+	—	..	.....	
7	H	..	+	—	..	.....	
7	H	..	+	+	3	Killed	Operative inoculation; rectum tied
8	H	..	+	+	3	Killed	Operative inoculation; rectum tied
9	H	..	+	—	4	Killed	Operative inoculation; rectum tied
10	H	..	+	—	5	Died	Operative inoculation; rectum tied
11	H	..	+	—	5	Died	Operative inoculation; rectum tied
12	H	..	+	—	2	Died	Operative inoculation
13	H	..	+	—	4	Died	Operative inoculation
14	H	..	+	+	4	Killed	Operative inoculation
15	H	..	+	—	(few hours)	Died	Operative inoculation
16	H	..	+	—	6	Killed	Operative inoculation
17*	..	..	..	—	3	Killed	Operative inoculation; rectum tied
18*	..	..	..	—	3	Killed	Operative inoculation; rectum tied

Summary

Nonoperative inoculations with culture	21
Kittens	7
Infected	0
Operative inoculations with culture	11
Kittens	11
Infected	3
Total number inoculated with cultures	32
Total number of kittens	16
Total number infected	3

* Received injection of culture of ameba-free intestinal contents.

times and kitten 3 five times. Twice, cysts obtained on cultivations were given by mouth. With these exceptions, inoculations were made with motile forms by rectum. In spite of their youth, amebic dysentery did not develop in any cat of this group.

In the work with cultures of strain H, only trophozoites were used. These introduced into the colon by rectal injection gave negative results. When cultures of this strain were introduced directly into the cecum by means of a needle, amebic lesions developed in three of eleven kittens operated on. In one of the three the result was positive in spite of the absence of stasis, for the bowel of this kitten had not been occluded by ligature (table 4). Kitten 6 was given a rectal injection of the same strain, four times, with negative results, whereas, following operation, its intestinal content contained motile amebas, but intestinal lesions were not found (see study 3). Kitten 7 remained uninfected following four rectal injections of strain H, but became infected after the fifth, or operative, inoculation. Two kittens which served as controls for those operated on, and which had been given injections of cultures made from stools that were free from amebas, gave negative results.

Besides the control kittens, inoculation from culture was attempted thirty-two times in sixteen animals, with infection resulting in only three. Those in which infection resulted all fell in the operative group of eleven kittens. The incidence of infection was 27 per cent. In twenty-one nonoperative inoculations in seven kittens, infection did not occur.

In view of the lack of knowledge concerning the possibility of modifying the virulence of *Endameba histolytica,* and of the fact that injections into animals were not made with the stools from which the cultures of strains A and H were grown, it is impossible to conclude from the results of the experiments here described, that the addition of rice starch to the medium in which the amebas were grown had reduced their infectivity. It is evident from the operative results with strain H that it is still infectious for cats. In spite of the lack of conclusive evidence, the impression remains that rice starch does reduce the infectivity of artificially grown *Endameba histolytica,* at least as far as the effect on cats is concerned.

Inoculation of Animals with Material Derived from Human Beings (Table 5).—In preparation for the examination of stools of patients with parasites, as practiced at the Mayo Clinic (see study 1), magnesium sulphate is taken before breakfast on the day that the test is to be made. This may not be carried out in the case of a patient who at the time of examination has active diarrhea. Since the resulting stools for examination usually are fluid, only the history of the cases can indicate whether the fluid stool was usual for the time or was the result of the physic. In the light of the histories subsequently studied, it was found that only four of the ten patients whose stools were used for inoculation were having dysenteric symptoms at the time of examination and may not have taken a preliminary dose of magnesium sulphate (table 2).

Of the four stools from cases of active dysentery, only one failed to produce amebic dysentery in kittens. Of the three stools that produced amebic lesions, one infected one of three kittens, one infected one of two and one infected all of four. The patient (case 8) from whom the last stool was obtained is known to have taken no preliminary magnesium sulphate; the animals that were given injections of this

TABLE 5.—*Data Concerning Inoculation of Animals with Endameba Histolytica from Human Beings*

Animal Given Injection	Source of Material, Case	Cysts	Trophozoites	Amebic Lesions	Manner of Death	Length of Life After Last Inoculation, Days
19	6	..	+	—	.....	..
20	11	..	+	—	.....	..
20	15	..	+	—	.....	..
21	11	..	+	—	.....	..
21	15	..	+	—	.....	..
22	11	..	+	—	.....	..
22	15	..	+	—	.....	..
23	9	+	..	—	Died	16
24	9	+	..	—	.....	..
25	9	..	+	—	.....	..
25	12	..	+	—	.....	..
25	13	..	+	—	.....	..
26	9	..	+	—	.....	..
27	5	..	+	—	Killed	3
28	5	..	+	+	Killed	4
29	10	..	+	—	.....	..
18	10	+	..	—	.....	..
30	12	..	+	—	.....	..
30	13	..	+	—	.....	..
7	13	..	+	—	.....	..
6	13	..	+	—	.....	..
31	14	..	+	—	Died	2
32	14	..	+	—	.....	..
33	14	..	+	—	.....	..
34	14	..	+	+	Killed	9
35	8	..	+	+	Killed	13
36	8	..	+	+	Killed	17
37	8	..	+	+	Killed	18
38	8	..	+	+	Killed	18

Summary

Inoculations from positive stools of human beings	29
Kittens inoculated	23
Kittens infected	6

patient's stool were larger than normal, being about one-half grown. Two were returned to their cages as soon as the injection was made, whereas two were held head down for ten minutes following the injection. The period of incubation was long because of the size and presumable age of the kittens, and holding the kittens head down failed to modify the results.

Cysts from the stools of two human beings were used for the inoculation of kittens by feeding, two for each stool, with negative results in all.

Both cysts and motile forms from the stools of human beings were used for injection into twenty-three kittens. The injections were given by mouth and by bowel, a total of twenty-nine times, with infection resulting six times. This is an incidence of 26 per cent of kittens used and of 20.7 per cent of injections.

Inoculation of Animals with Stools of Infected Kittens.—Infected stools of nine kittens were used as material for injection into ten kittens a total of fourteen times, with positive results in three (table 6). Kitten 32 was given injections four times, once of material from kitten 7 and three times of material from kitten 34. In this case, the period of

TABLE 6.—*Data Concerning Inoculation of Animals with Positive Stools from Kittens*

Kitten Given Injection	Source of Material, Kitten	Amebic Lesions	Manner of Death	Length of Life After Last Inoculation, Days
39	28	+	Killed	3
40	28	—	Killed	6
41	26	+	Died	2
9	26	—	.....	..
38	25	—	.....	..
42	7	—	.....	..
32	7	—	.....	..
32	34	—	.....	..
32	34	—	.....	..
32	34	+	Killed	5
43	36	—	.....	..
43	38	—	.....	..
44	37	—	.....	..
25*	27	—	.....	..
25*	40	—	.....	..
25*	40	—	.....	..

Summary

Inoculations with positive stools of kittens	14
Kittens inoculated	10
Kittens infected	3

* Given injections of fecal material from kittens which had been inoculated with Endameba histolytica and in which diarrhea had developed but lesions had not.

incubation must be indefinite, for any one of the last three injections may have produced the disease. The infection from kitten to kitten was not carried beyond the third subpassage in any case. These data are in marked contrast with those of Sellards and Baetjer,[59] who carried a strain from animal to animal for eleven generations; of Dale and Dobell, who carried a strain for forty-three passages, and of Mayer, who carried strains through animals for thirty-two, thirty-nine and fifty-nine passages, respectively. In my work, however, as already explained, no animal was killed for the sake of the infective intestinal material.

In the ten infected kittens, the stools of which could be examined during life for diagnosis, the period of incubation varied from one day to nine days, with an average of five days (table 7). The period of incubation was shorter in the younger cats.

SUMMARY OF STUDY 2

Cultures of strains of *Endameba histolytica,* not previously tested for virulence, failed to infect kittens, except in those instances in which the infective material was introduced operatively directly into the cecum of the experimental animal. The proportion of animals infected by this last means was 27 per cent.

No animal was infected by the use of stools from patients free from symptoms of dysentery. The stools of all but one of the patients who presented symptoms of active dysentery caused infection; the results in six of fourteen experiments were positive, an incidence of 42.8 per cent.

Thirty per cent of animals that received injections of the infected stools of cats that were ill with amebic dysentery became infected. Survival of the infection beyond the third subinoculation did not occur.

TABLE 7.—*Incubation Period in Animals Infected with Endameba Histolytica* *

Kitten	Source of Material	Incubation, Days
14	Culture strain H	3
28	Stool of human being	3
35	Stool of human being	7
36	Stool of human being	6
38	Stool of human being	9
37	Stool of human being	9
41	Stool of kitten	1
42	Stool of human being	6
32	Stool of kitten	3
39	Stool of kitten	3

* Of the animals represented in this table, kitten 14 was infected by operation; all others by rectal injection. Kittens in which inoculation was by operative methods, and in which the rectum was tied, are not included in this table.

The use of cysts for inoculation, both those from culture and those from the stools of human beings, gave negative results.

The effect of passage through animals on the virulence of *Endameba histolytica* could not be determined.

Evidence is not presented to show that the resistance of the experimental animal to *Endameba histolytica* is increased by previous attempts to infect it with this organism.

(To be Concluded)

BASAL CELL CARCINOMA

A STUDY OF EIGHT HUNDRED AND THIRTY-SIX CASES *

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The term basal cell carcinoma is used to designate carcinoma arising from the basal cell layer of the epidermis and retaining to a large extent the characteristics of basal cells. Reports in the literature indicate that primary basal cell carcinoma of the mucous membrane is not common.

The work presented was undertaken for the purpose of determining, if possible, whether or not basal cell carcinoma, with its low grade of malignancy, occurs primarily on the mucous membranes, and to study the relation of the basal cell carcinoma having its origin on the skin to the so-called basal cell carcinoma having its origin on the mucous membrane. A recent report of two cases of basal cell carcinoma of the cervix suggested the possibility of a highly malignant squamous cell carcinoma being confused with a basal cell carcinoma. I had opportunity to study the tissues from these cases, and I believe that the report in question concerns active squamous cell carcinomas. In both cases, the history was that commonly noted in cases of highly malignant squamous cell carcinoma. In one case there was extensive pelvic metastasis, and the patient lived less than nine months after the onset of symptoms; in the other case, the patient lived less than twenty-two months after the diagnosis had been made.

REVIEW OF THE LITERATURE

Basal cell carcinoma, or rodent or jacobean ulcer, was first described by Jacob [1] in 1827. His description has not been essentially modified: slow growth, peculiar condition of the edges and the surface of the ulcer, absence of contamination of the neighboring lymph nodes and distribution in the region of the eyes. Following this report, Hutchinson,[2] in 1860, reported a clinical case in which the lesion occurred on the skin of the groin. Until this time basal cell carcinoma had been considered peculiar to the face.

Krompecher,[3] in 1900, reported his histologic observations on basal cell carcinoma. He concluded that the origin is in the basal cell layer

* Submitted for publication, April 22, 1930.

* Work done in the Section on Surgical Pathology, the Mayo Clinic, Rochester, Minn.

1. Jacob, Arthur, quoted by Little: Brit. J. Dermat. **27**:145, 1915.

2. Hutchinson, J.: Rodent Ulcer of the Forearm, Tr. Path. Soc., London **48**:220, 1860.

3. Krompecher, E.: Der drüsenartige Oberflachenepithelkrebs, Beitr. z. path. Anat. u. z. allg. Path. **28**:1, 1900.

of the epidermis, and he suggested the classification of cutaneous carcinomas into basal cell and spinal cell carcinomas. This has been widely accepted. Following the work of Krompecher, the growths became known as basal cell carcinoma, or epithelioma. Borrman [4] opposed this theory that basal cell carcinoma has its origin in the basal cell layer of the epidermis, and maintained that it originates in cell rests and becomes united to the epidermis only secondarily.

Cohnheim [5] was of the opinion that in the early stage of embryonic development more cells are produced than are required for building up the part concerned, so that there remains unappropriated a quantity of cells that because of their embryonic character are endowed with a capacity for proliferation. Furthermore, the early slow growth is due to the poor blood supply, and as soon as the lesion penetrates the deeper tissues and receives a better blood supply, growth is more rapid.

Walker,[6] Mallory,[7] Paul,[8] Johnson [9] and others believed that basal cell carcinoma has its origin from hair follicles, sebaceous glands, sweat glands or a combination of these. Blasdell [10] believed that it has its origin in seborrheic patches or senile patches of the skin, and Ewing,[11] that it arises perhaps exclusively from the basal cells, often from misplaced and embryonal group of such cells, but the normal transformation into squamous cell entirely fails.

Cleveland and Paul,[12] in 1920, stated that the type of epiblastic development depends on the situation of the cells. They gave as an example the epiblast covering the chorionic villi, which forms a syncytium and Langhans' layer of cells, while other cells similarly derived form the epidermis on the surface of the body. That is, in spite of the uniformity of origin and presumed equality of potentialities, the highest development of the cells depends on the structures among which they find themselves situated in regard to the whole body.

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4. Borrman, Robert: Die Entstehung und das Wachstum des Hautcarcinoms, Ztschr. f. Krebsforsch. **2**:1, 1904.

5. Cohnheim, J. F.: Lectures on General Pathology (Translation), London, The New Sydenham Society, 1889-1890, vol. 2, p. 760.

6. Walker, Norman: The Pathology of Rodent Ulcers, Tr. Path. Soc. London **45**:172, 1894.

7. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, pp. 371-373.

8. Paul, Norman: Observations on the Origin, Causation and Treatment of Rodent Ulcer, M. J. Australia **1**:85, 1923.

9. Johnson, F. H.: The Treatment of Rodent Ulcers (Basal Cell Epithelioma), with Especial Reference to Recurrence, Lancet **1**:389, 1926.

10. Blasdell, H. E.: Basal Cell Carcinoma, J. Kansas M. Soc. **23**:338, 1923.

11. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1919, p. 457.

12. Cleveland, J. B., and Paul, Norman: Rodent Ulcers and Allied Growths: An Analysis of Sixty Australian Cases, M. J. Australia **1**:407, 1920.

Kyrle [13] explained the failure of the cells of basal cell carcinoma to differentiate and become keratinized as normal basal cells do on the basis that the basal cell layer of the skin has two tasks to fulfil: to differentiate into pavement epithelium and to act as the matrix for the postfetal sebaceous glands without formation of squamous cells. It follows that when tumors arising from the basal cell layers differentiate into pavement epithelium, squamous cell carcinoma is formed. When the tumor arises from the cells acting as the matrix for postfetal sebaceous glands, basal cell carcinoma is formed.

Lunford and Taussig [14] reported 230 cases of superficial carcinoma, 89 of which were basal cell and 141 squamous cell. They made separate analyses of the basal cell and squamous cell types of carcinoma and further divided them into those occurring on the skin and those occurring on the mucous membrane. Of 109 carcinomas of the skin, 89 (81.65 per cent) were basal cell and 20 (18.25 per cent) were squamous cell. The 121 carcinomas of the mucous membrane were of the squamous cell type.

In 194 cases of carcinoma of the tongue that I recently reported,[15] I did not observe a basal cell carcinoma.

Martzloff [16] studied 387 cases of carcinoma of the cervix and concluded that basal cell carcinoma with its low grade of malignancy does not occur in the cervix. He divided carcinomas of the cervix into three groups, basing the classification on cell differentiation: (1) spinal type of cancer cell, (2) transitional type of cancer cell and (3) the fat spindle type of cancer cell. Martzloff believed that the last type corresponds to Krompecher's [17] basal cell carcinoma, but in his series it proved to be the most malignant.

There is a striking similarity between the observations of Broders [18] and those of Martzloff in carcinoma of the cervix. In Martzloff's series, 47 per cent of the patients with spinal cell carcinoma, 24.2 per cent with the transitional type of carcinoma, and 9.5 per cent with fat spindle cell type of carcinoma remained well for five years after operation. These

13. Kyrle, J.: Beitrag zur Frage der Basalzellengeschwülste der Haut, Arch. f. Dermat. u. Syph. **121**:246, 1916.

14. Lunford, C. J., and Taussig, Laurence: Superficial Epitheliomata, California & West. Med. **25**:740, 1926.

15. Owen, May: Lesions of the Tongue with Special Reference to Their Location, Texas State J. Med. **22**:693, 1927.

16. Martzloff, K. H.: Cancer of Cervix Uteri, Variations in Malignancy of Different Varieties, Northwest Med. **25**:127, 1926.

17. Krompecher, E.: Zur vergleichenden Histologie der Basaliome, Ztschr. f. Krebsforsch. **19**:1, 1922-1923.

18. Broders, A. C.: The Grading of Cancer and Its Practical Application, Arch. Path. **2**:376, 1926.

correspond, respectively, to Broders' cases graded 2 with good results in 53.33 per cent, those graded 3 with good results in 21.56 per cent and those graded 4 with good results in 9.52 per cent. None of these types conformed to the basal cell carcinoma of the skin as regards lack of malignancy.

Vinson,[19] in a series of 159 cases of carcinoma of the esophagus, did not see a basal cell carcinoma. In all except two cases the malignancy was graded high. In the two cases of low grade malignancy the neoplasm occurred on old strictures which had been caused by lye and which had been repeatedly dilated. They corresponded more or less to carcinoma experimentally produced by tar, which is often of a low grade of malignancy. So far as I am aware, basal cell carcinoma has not been produced experimentally.

Contrary to most other authorities, Krompecher [20] reported that in his observation basal cell carcinoma originates on the mucous membrane of the uterus, esophagus, larynx, nose and sinuses. He described the neoplasm in these situations as forming long, narrow, concave bands with indefinite borders. This he considered as being a separate type of basal cell carcinoma. He also believed that the tumors described by a number of pathologists as endotheliomas, cylindromas and mixed tumors of the salivary glands are forms of basal cell carcinoma. According to his observations, about half of the malignant tumors of the nose and a third of those of the larynx correspond to the basal cell type; the nose is the most common site (twenty-nine of sixty) and the larynx is the next most common (fifteen of fifty). However, taking them as a whole, he has found the occurrence of basal cell carcinoma much more rare on the mucous membrane than on the skin.

New [21] and Figi [22] never observed that a basal cell carcinoma originated on the mucous membrane of the trachea, larynx, mouth, nose or sinuses.

Broders,[23] in his report of 362 cases of carcinoma of the cavities and internal organs of the head and neck, did not mention a basal cell carcinoma. He studied the clinical and pathologic aspects of carcinoma thoroughly and divided the lesions into groups according to their degree of cell differentiation.[24] In the microscopic study of thousands of

19. Vinson, P. P.: Personal communication to the author.

20. Krompecher, E.: Zur Kenntnis der Basalsellenkrebse der Nase, der Nebenhohlen, des Kehlkopfes und der Trachea, Arch. f. Laryngol. u. Rhinol. **31**:443, 1918.

21. New, G. B.: Personal communication to the author.

22. Figi, F. A.: Personal communication to the author.

23. Broders, A. C.: Epithelioma of Cavities and Internal Organs of the Head and Neck, Arch. Surg. **11**:43, 1925.

24. Broders, A. C.: Squamous-Cell Epithelioma of the Lip: A Study of Five Hundred and Thirty-Seven Cases, J. A. M. A. **74**:656, 1920.

carcinomas, he never observed a true basal cell carcinoma of the jacobean type having its origin on the mucous membrane.

DATA IN EIGHT HUNDRED AND THIRTY-SIX CASES

The work reported here is based on the microscopic study of 836 cases diagnosed basal cell carcinoma, which were observed in the Mayo Clinic from August, 1915, to January, 1927. In every case, the diagnosis was made on the basis of a microscopic examination of excised portions or the whole lesion. Special effort was made to ascertain the site of the original growth and to obtain tissue for study. Those cases in which tissue was not available and those in which there was any doubt as to the site of the original growth were discarded. I also made a careful microscopic examination of more than 500 specimens removed at biopsy from the cavities and internal organs of the head and from the neck, esophagus, bronchi, cervix uteri, bladder and rectum; in no case did I observe a carcinoma that conformed to the characteristics of basal cell carcinoma or rodent ulcer of the skin. The cases will not be reviewed in detail; a summary of some of the more general observations follows:

The average age of the patients was a little more than 56 years; the youngest patient was aged 18 and the oldest 81. The condition was preponderantly one of the fourth, fifth and sixth decades. In this series, basal cell carcinoma was slightly more than twice as common in men as in women. In 774 cases (92.58 per cent), the lesion occurred on the face and head; in 226 cases (27.03 per cent), on the nose. Seven hundred and eighteen of the lesions were simple basal cell; 111 were basal cell and squamous cell mixed, the squamous cell being graded 1 and 2 (fig. 1), and 7 were pigmented basal cell carcinomas. It may be this pigmented type that has led a few observers to consider the nevus as the origin of the basal cell carcinoma.

The material for the study of these 836 cases included only specimens removed at operation. Three of these neoplasms, although resembling basal cell carcinoma, proved, on careful microscopic study, to be highly malignant squamous cell carcinoma; 1 was from the mucous membrane of the nose and 2 from the cervix. As stated, on cursory microscopic examination, these three cases closely resembled basal cell carcinoma, but on more careful study they were found to be squamous cell carcinoma graded 3 or 4 (fig. 2). The tumors were almost wholly of undifferentiated cells containing many irregular mitotic figures and varying greatly in size, shape and number of nuclei. The patient in each case gave a history of a tumor of short duration and extensive metastasis, which corroborated the microscopic data.

I was impressed with the frequency with which the tumors graded 3 and 4 grew in the form of solid branching strands of closely packed

cells, and how readily the neoplasm could be mistaken for basal cell carcinoma. The cells were rather less regular in form and character of nuclei than is usual in carcinoma of the skin. The clinical course of the highly malignant squamous cell carcinoma is more rapid than that of

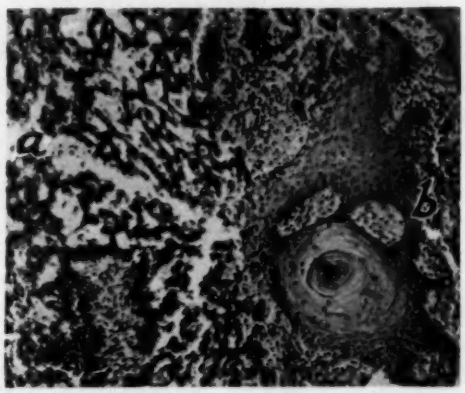

Fig. 1.—Mixed basal cell and squamous cell carcinoma (× 60); *a*, basal cell carcinoma; *b*, squamous cell carcinoma; of malignancy graded 1.

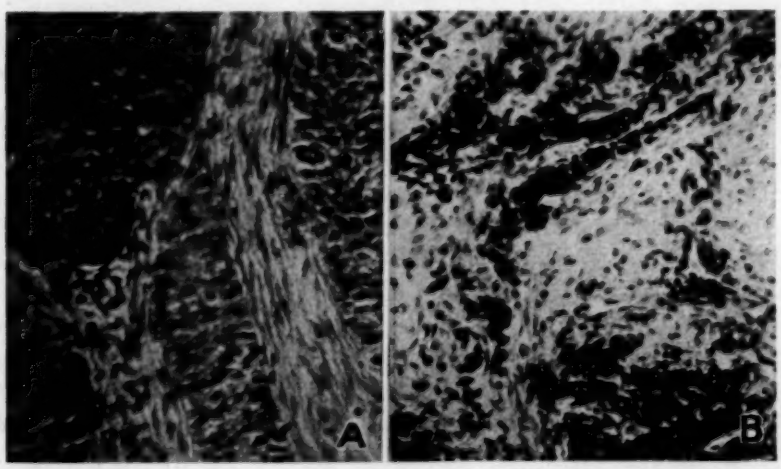

Fig. 2.—*A*, squamous cell carcinoma of the cervix, originally diagnosed basal cell carcinoma (× 100); malignancy graded 4. *B*, squamous cell carcinoma from the mucous membrane of the nose, originally diagnosed basal cell carcinoma (× 100); malignancy graded 4. The patient had extensive metastasis.

the basal cell carcinoma; there is often metastasis, and the cells are only slightly differentiated.

The basal cell carcinomas studied, besides the familiar rodent ulcer, comprised many flattened, irregular patches and smooth, elevated,

rounded masses. Most of them were characterized by slow growth, smooth pearly borders and granular bases often covered by a dirty-brown crust. In none of the cases were the lymph nodes involved. As a rule, the lesions occurred singly, but they were sometimes multiple. Although the lesion was carcinomatous in structure, the cells did not penetrate far into the deeper tissues.

The structure of these growths varied greatly. The stroma was either abundant or scanty. The relatively bulky compact masses of cells, connected by narrow strands of similar cells, lay in artificial spaces, from which they readily shrank on hardening. There was a tendency to concentric arrangement of the cells which formed the masses, the greater percentage of which showed a marginal palisade or a layer of columnar-like cells. The large masses often contained clear areas in which were small amounts of degenerating mucus. In another type, the growth assumed more the appearance of finger-like processes, often separated into lacunae that suggested in general glandular carcinoma or adeno-carcinoma.

The tumor cells resembled the basal cells of the epidermis, and were small and polyhedral or spindle-shaped, and one, two or more layers thick. These cells were characterized by a cylindric or spindle shape, a small amount of cytoplasm, a large amount of nuclear material, which took the shape of the cell and stained much more intensely than the average squamous cell, and by the intimate relation of the cells to each other.

It has been generally believed that basal cells do not possess prickles or bridges. Broders,[25] however, was convinced that they do. Mallory stated that rarely is there a hint of the formation of the intercellular bridges or prickles. I studied a number of specially prepared sections and am able to corroborate Broders' observations. In order to demonstrate satisfactorily the intercellular bridges or prickles in basal cell carcinoma, it is necessary to cut the sections from 2 to 3 microns thick and stain them carefully. With the usual technic, the cells are too closely packed to permit the finest cytologic studies. Because of the presence of prickles in some cases in both basal cell and squamous cell carcinoma, it is sometimes difficult to differentiate these two types.

Mitosis is often found in basal cell, as well as in squamous cell, carcinoma. However, there is a question as to its relative significance in basal cell carcinoma.

COMMENT

The division of carcinomas into squamous cell and basal cell forms, as well as into carcinoma of the mixed tumor type and adenocarcinoma,

25. Broders, A. C.: Personal communication to the author.

is of more than academic interest. The distinguishing features are determined by the amount of cell differentiation. Another feature of this cellular classification is that a particular growth may not be entirely of one type but may be mixed. Particularly is this true of the basal cell and squamous cell carcinoma. It is only natural that this should be so, since the normal cells from which carcinomas spring are not widely separated, nor do they differ widely in their nature. Montgomery [26] recently called attention to the mixed basal cell and squamous cell or transitional carcinoma and applied to this the term basal-squamous cell carcinoma. He reported fifteen cases in which he showed that in the transitional forms the prognosis is serious as compared with that in the basal cell carcinoma.

Since a high percentage of carcinomas described by some writers as basal cell arising from the mucous membrane of various organs, such as the cervix, esophagus, mouth and sinuses, metastasize, if the grade of malignancy is not too low, and since they grow more rapidly than basal cell carcinoma of the skin, the generalization seems justifiable that if epithelial tumors can be definitely identified as forming pearls or if they grow rapidly and destructively and metastasize, and consist to a large extent of undifferentiated cells, they may not be considered basal cell carcinoma. It is well known that in basal cell carcinoma, if the cells once gain access to mucous surfaces, they rapidly involve most, or all, of the exposed surfaces, and a relatively large tumor results without metastasis.

Early, as well as recent, writers have been unanimous in their belief that the basal cell carcinoma arises from the basal cell layer of the epidermis or its appendages, and almost all have considered the logical necessity of selecting some point of origin. Few have contended that the basal cell carcinoma also develops from the basal cell layer of the mucous membrane. My own studies induce me to attribute its origin to the basal cell layer of the skin. In this series of 836 cases, I was unable to find a true basal cell carcinoma having its origin on the mucous membrane. However, a number of growths had extended from the skin to the mucous membrane, particularly those near the eyes and nose. In some cases there was even extensive destruction of the orbit and its contents.

SUMMARY

Since Jacob, in 1827, first described basal cell carcinoma, or rodent ulcer, reports and studies of these tumors have appeared with comparative frequency. Basal cell carcinomas have been confused microscopic-

26. Montgomery, Hamilton: Basal-Squamous-Cell Epithelioma, Arch. Dermat. & Syph. **18**:50, 1928.

ally with nevi, endotheliomas, sarcomas, carcinomas (mixed tumor type) and highly malignant squamous cell carcinomas. Certain of the more recent writers have been unable to confirm the observations of Krompecher and others with regard to the occurrence on the mucous membrane of basal cell carcinoma with its low grade of malignancy.

My observations have been similar in many respects to the observations of others, but a recent report of two cases of basal cell carcinoma of the cervix suggested a review of the entire subject. However, my study of 836 cases of basal cell carcinoma seems to show that carcinomas having their origin on the mucous membrane lack the benign characteristics of basal cell carcinomas, or rodent ulcers of the skin. I have emphasized the contrasting characteristics of the basal cell carcinoma, or rodent ulcer of the skin, and the so-called basal cell carcinoma with its origin on the mucous membrane.

CONCLUSIONS

My observations do not indicate that true basal cell carcinoma may occur on the mucous membrane.

The review of cases in this paper lends weight to the contention that carcinomas having their origin on the mucous membrane lack that low grade of malignancy which the basal cell carcinomas that are situated on the skin possess.

It would seem rather hazardous to diagnose as basal cell carcinoma a tumor having its origin from the basal cell layer of the mucous membrane when there are present metastasis and rapid extensive growth consisting chiefly of undifferentiated cells or cells showing squamous characteristics. This tumor seems to be a squamous cell carcinoma.

NEUROMUSCULAR CHANGES IN AMYELIA AND THEIR RELATION TO THOSE OF CONGENITAL CLUBFOOT *

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Although there is a wide gap between anatomic conditions in amyelia and those in congenital clubfoot, there is nevertheless a remote relationship between the two which indicates that the difference in their construction is merely one of degree of involvement. Congenital clubfoot has at various times been considered a deformity brought about by a neuromuscular imbalance of the foot, manifested by a predominance of certain muscle groups over their antagonists. This conception has been largely the result of clinical observation, although a considerable amount of evidence of structural changes in the neuromuscular mechanism has been contributed by anatomic investigation. In a recent article,[1] I presented evidence to show that congenital clubfoot may be produced by an abnormal development of the peroneal nerve. This structure has certain embryologic peculiarities by which during intrauterine development it may be exposed to harmful influences that are instrumental in preventing the normal outgrowth of a portion or all of its constituent nerve fibers as they proceed from the spinal cord. As a direct consequence of a loss of nerve substance to the muscles supplied by these nerves, a deficiency occurs in the synergy of opposing muscle groups controlling the position of the foot.

In his discussion of amyelia, Ernst [2] stated that practically all malformations of the central nervous system are the results of varying degrees of the same disturbance in development, namely, a deficient anlage of the medullary plate or an incomplete closure of the medullary groove. With complete absence of the cord, the musculature of the trunk and the extremities may be present and even supplied with nerves. The muscles can develop completely without the influence of the medullary canal, and only in extra-uterine life do they become dependent on the motor nervous system. That the musculature can develop normally without the motor nerves has also been shown experimentally in frogs by removing the anlage of the brain and cord.

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* Submitted for publication, May 24, 1930.

* From the Pathological Laboratory of St. Francis Hospital.

1. Dittrich, R. J.: Pathogenesis of Congenital Club-Foot (Pes Equinovarus): An Anatomical Study, J. Bone & Joint Surg. **12**:373, 1930.

2. Ernst, P.: Störungen der Entwicklung, in Aschoff, L.: Pathologische Anatomie, ed. 2, Jena, Gustav Fischer, 1911, vol. 2.

The object of this study was to investigate the condition of the muscles and nerves of the lower extremities in amyelia and to determine in what respects they differ from those found in congenital clubfoot.

DESCRIPTION OF SPECIMEN

The specimen studied was a new-born girl with multiple malformations. The child was born of a first pregnancy. The date of the mother's last menstrual period was May 9, 1929. Fetal heart sounds were last heard on Dec. 24, 1929, and delivery occurred on Dec. 26, 1929. The history stated that no roentgen or radium treatments had been given to the mother.

The skin of the specimen was dark and somewhat macerated. In some areas, the discoloration of the skin indicated hemorrhage. This was most marked on the dorsum of the right foot. The head showed a marked defect of the posterior portion (anencephaly). The upper extremities appeared normal. There was an eventration of the anterior abdominal wall allowing the contents to protrude. The spine showed marked kyphosis in the dorsal region, and the skin over this area was absent, the result evidently of pressure necrosis. The muscles of this region were small and poorly developed. The right foot was in a position of equinovarus, and the left foot in a position of calcaneovalgus. The left leg was larger than the right.

Examination of the muscles of the legs showed that the anterior muscles of the left leg were larger and better developed than those of the right leg. This was most noticeable in the tibialis anterior; less so in the extensor hallucis and the extensor longus digitorum. The peronei on each side were not clearly differentiated and appeared to be fused. The posterior muscles were about equal in development and volume on the two sides. The right tibialis anterior, in addition to being smaller than the left, was distinctly pale and consisted of fine fasciculi, which were easily separated.

In the thighs, both portions of the sciatic nerve were present and no difference between the nerves on the two sides was shown. They were grayish brown. The peroneal nerve on the right side was followed down into the leg and was seen to give off branches to the muscles. The branches to the muscles were fine filaments and were more numerous than ordinarily.

On both sides, the cutaneous portions of the superficial peroneal nerves were present and had a normal glistening appearance.

The spinal column consisted of the vertebral bodies with small rudimentary pedicles projecting only a few millimeters from the bodies. There was no trace of a spinal cord from the upper dorsal to the midsacral segments. Several of the vertebral bodies were found to have well formed centers of spongy bone.

Microscopic Observations.—The controls used in this investigation were the same as those used in the determination of the anatomic condition of the muscles in a case of clubfoot.[1] This was necessitated by the impossibility of finding a suitable control in a case of amyelia. The muscle from which control sections were made was the extensor carpi ulnaris.

The appearance of certain muscles of the legs was studied by means of the hematoxylin-eosin staining method.

The right peroneus longus showed a general narrowing of the muscle fibers and an increase in the number of nuclei. All fibers showed a moderate loss of sarcoplasm and occasionally fragmentation. Cross-striations were present in all fibers. Many small hemorrhages were found in the muscle tissues. Numerous dilated blood vessels were noted throughout the section.

A section of the right gastrocnemius showed an increase in the number of nuclei, a general narrowing of the fibers and a moderate loss of sarcoplasm. Cross-striations were present in all the fibers, although in some they were indistinct. Fragmentation was noted in some fibers, and numerous small hemorrhages were seen. There was an increase in the number of blood vessels. These also were dilated.

The right tibialis anterior showed a slight increase in the number of nuclei. Considerable variability was noted in the size of the fibers, some being normal but most of them being narrowed. Cross-striations were present in all fibers, but loss of sarcoplasm was not as marked as in the other muscles of the right leg. A few small areas were present in which there was complete loss of muscle substance and replacement by fibrous tissue. Several small hemorrhages were noted. The blood vessels were dilated but were not increased in number.

The left peroneus longus showed a marked increase in the number of nuclei and a distinct loss of sarcoplasm. Cross-striations could be seen in all fibers, although in some they were not clear. Individual fibers showed fragmentation. Numerous small hemorrhages were present. Blood vessels were increased in number and dilated.

The left gastrocnemius disclosed wide variation in the appearance of the fibers. Most fibers were narrowed, at times to one third or one fourth of their normal width, and showed an increase in the number of nuclei, together with loss of sarcoplasm. Cross-striations were present in all fibers, but indistinct in some. There were numerous small areas of hemorrhage and many individual red blood cells scattered throughout the muscle. Among the atrophied fibers were some which were more normal in appearance. These showed a more intense staining reaction, clear cross-striations and no increase in the number of nuclei, and were approximately normal in size (fig. 1).

In the left tibialis anterior, most of the fibers were slightly narrower than normal. No increase in the number of nuclei and only a mild loss of sarcoplasm were noted. Cross-striations were normal. There was no increase in the number or dilatation of blood vessels.

Microscopic examination of the nerves was limited to a study of cross-sections of the sciatic nerves, the specimens being obtained at the level of the distal portions of the thighs. The nerve tissues were embedded in gelatin, sectioned and stained with hematoxylin and eosin and by the Spielmeyer methods.

The left sciatic nerve was larger than the right.

The most striking feature of the nerve tissues was the large number of blood vessels. These were dilated, frequently having a thin wall, and were well filled. Hemorrhages of variable extent were found in both sections, mostly in the epineurium and the perineurium (fig. 2).

By means of the hematoxylin-eosin staining method, numerous sheath cells were seen, which had a normal appearance. No fibrosis, sclerosis or degenerative changes were noted. With the Spielmeyer staining method, the nerves showed a rather uniform distribution of myelin, although myelinization was incomplete.

ANALYSIS OF ANATOMIC CHANGES

The alterations in the muscles were mostly such as would be expected in beginning simple atrophy. They consisted almost entirely of narrowing of the fibers, an increase in the number of nuclei and loss of sarcoplasm. The specific fiber structure was retained, and cross-striations

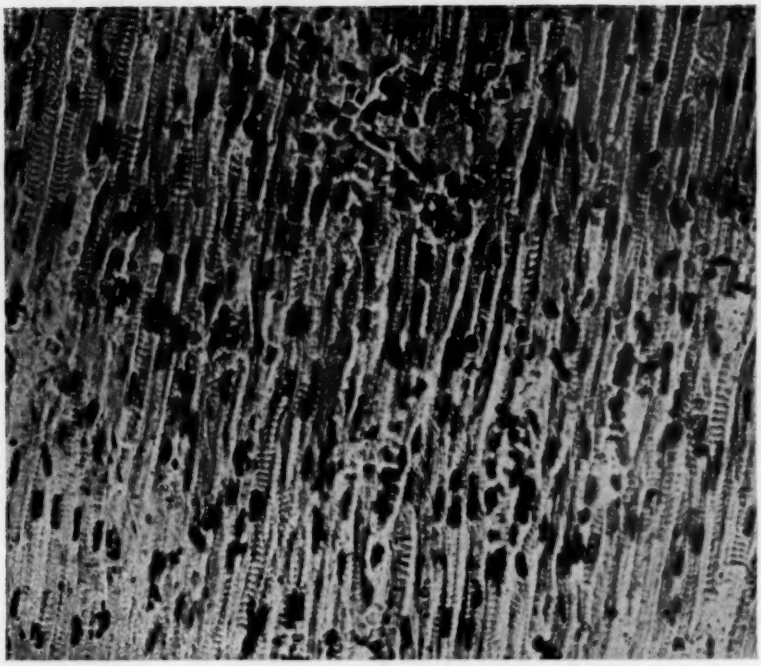

Fig. 1.—High power photomicrograph of the left gastrocnemius, showing variation in size of fibers and loss of sarcoplasm.

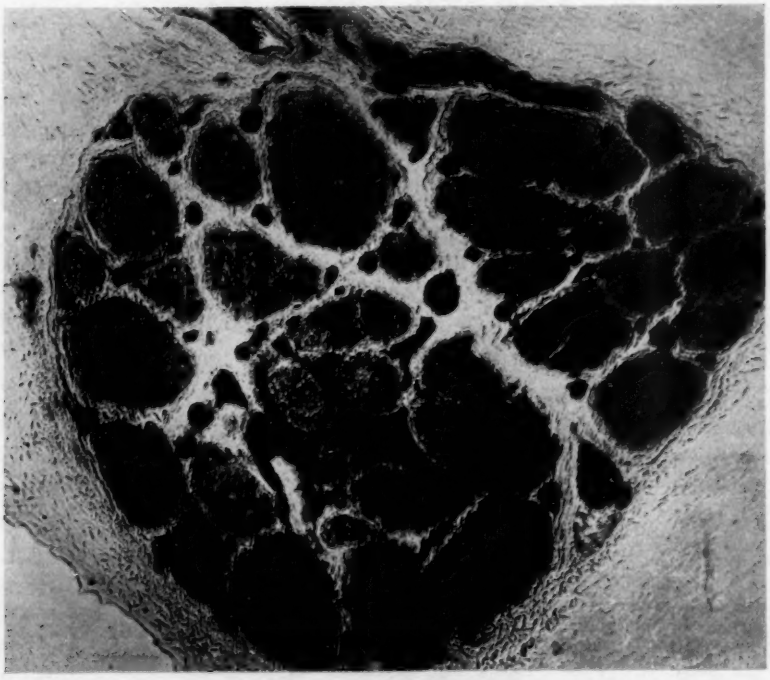

Fig. 2.—Low power photomicrograph of left tibial and peroneal nerves, showing degree of myelinization, hyperemia and hemorrhage.

were seldom lost. The only evidence of degenerative atrophy consisted of a few small areas in which the muscle structure had entirely disappeared and was replaced by fibrous tissue.

From a study of the condition of nerves and muscles, there is no reason to believe that the position of the feet was in any way dependent on the functional activity of the different groups of muscles of the leg. On the right side, with the foot in a position of equinovarus, the atrophic process was approximately equal in the three muscles examined. It may be significant that on the left side, with the foot in a position of calcaneovalgus, the tibialis anterior was grossly proportionately larger than the other muscles, and that microscopically it had a more normal appearance. However, in the absence of connections with the central nervous system, it is not likely that the position of either foot was produced by muscular activity. The position of the feet is interpreted as the result of mechanical influences, such as pressure conditions in utero or whatever effect may have been produced by the pull of the inactive muscles.

A simple atrophy of the muscles as seen in this case is in distinct contrast with the degree of atrophy found in congenital clubfoot, in which, even at the same stage of development, the atrophic process is more pronounced, at times proceeding to a complete disappearance of muscle structure and to fibrous degeneration. The difference in the degree of atrophy may be attributed to the fact that, in one case, there is no muscular activity whatever, whereas, in the other, certain muscles are under normal nervous control. As a result of the action of the normal muscles, the denervated muscles are more or less continually subjected to strains, which would accelerate the atrophic process.

The pathologic changes in the nerves were clearly not extensive. With the exception of vascular changes—hemorrhage and hyperemia—the nerves presented a normal appearance, if it is borne in mind that the incomplete myelinization was normal for this stage of development of the fetus.

The occurrence of hemorrhage in association with congenital abnormalities was repeatedly emphasized by Bagg [3] in his investigations of the effects of roentgen rays on descendents of irradiated white mice. He described the formation of localized blood clots in the portions of the extremity which subsequently showed various types of deformity, such as clubbing, syndactylia, polydactylia or amputation. Bean,[4] who made an analysis of the anatomic conditions of such deformities in white mice, concluded that the underlying disturbance was a deficiency

3. Bagg, H. J.: Hereditary Abnormalities of the Limbs, Their Origin and Transmission, Am. J. Anat. **43**:167, 1929.

4. Bean, A. M.: A Morphological Analysis of Foot Abnormalities Occurring in Descendents of X-Rayed Mice, Am. J. Anat. **43**:212, 1929.

in the blood vessel and nerve supply to these regions. The influence of muscle action was considered as a factor in the production of the deformity, although it was evident that this could not explain all the features.

Hyperemia as seen here in the nerves and muscles was described by Kiyono [5] as a common observation in the endocrine glands in cases of anencephaly. As it has not been found in the muscles and nerves in congenital clubfoot, it seems that it is closely related to extensive defects of the central nervous system.

With reference to changes in muscles and nerves as seen in congenital clubfoot, it will be recalled that certain muscles frequently show alterations consisting of varying degrees of atrophy. The nerves, on the other hand, when subjected to anatomic examination in practically all instances, have failed to reveal any distinct abnormality, although there is a decided diminution in electrical excitability. In order to ascribe deformities of the feet, such as clubfoot, hollow foot, etc., to remote disturbances, such as malformations in the lower part of the spine, it is necessary to consider the medium by which such long-range effects may be brought about. An association of deformities of the foot with defects in the lower part of the spine would require only one factor to be considered—an interference with the normal development or function of the nerves in the lumbar and sacral regions. As a rule, pathologic changes are lacking in the nerves supplying the muscles that are definitely atrophic—a factor which has led to the conclusion that there is merely a physiologic interruption of continuity. The absence of anatomic abnormalities in the nerves may rightly militate against any conception that deformities of the foot are the results of developmental defects in the lumbosacral spine. However, in view of the fact that a similar appearance of the nerves may accompany complete amyelia, as seen in this case, it would be difficult to contradict the possibility of this mode of development solely on the ground of negative changes in the nerves. The observations indicate that although visible structural changes are not present in the nerves, they may be functionally deficient. Also the question is raised as to why there should not be more extensive degenerative changes in the peripheral nerves, since following section of a nerve wallerian degeneration begins in a few days.

Another important question suggested by this case is that of myelodysplasia, a congenital aplasia or hypoplasia of a portion of the spinal cord, a condition frequently used as an explanation for the occurrence of motor, sensory, vascular and trophic disturbances of the lower

5. Kiyono, H.: Die pathologische Anatomie der endokrinen Organe bei Anencephalie, Virchows Arch. f. path. Anat. **257**:441, 1925.

extremities. While a case of amyelia at first sight may appear to be an example of myelodysplasia in its more severe form, it is necessary to distinguish between primary congenital defects due to intrinsic disturbances and secondary degenerative processes due to extrinsic influences. It will be recalled that in the case under investigation the peripheral nerves were fully developed even to the point of myelinization. This, according to the outgrowth theory of His, indicates that the spinal cord was present in the early embryonic stages for a period of time sufficient to allow the nerves to develop. Also in this specimen there was a complete absence of the laminar arches and of the pedicles of the vertebrae, which ordinarily serve as a protective covering for the spinal cord. Under these conditions, it is apparent that the absence of the spinal cord was due to a pressure necrosis as a result of lack of protection. This leads to the conclusion that the primary defect of development was that of the osseous portions of the spinal canal. This seems to harmonize with the idea that in cases of congenital clubfoot resulting from interference with the development of certain nerves in the lumbosacral area, the primary influence is a defect in the bony structures.

SUMMARY

A study of the muscles of the lower extremities in a case of amyelia indicates that the muscular alterations are similar to those found in congenital clubfoot, with the exception that the changes are not so far advanced. The appearance of the sciatic nerves in amyelia is similar to that of the nerves in congenital clubfoot, with the addition, in the former, of a marked degree of hyperemia. The relationship of the spinal canal in amyelia to the spinal cord indicates that the primary defect of development is that of the bony portions of the spine.

THE CHANGES OF THE SPLEEN IN SUBACUTE BACTERIAL ENDOCARDITIS *

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The copious literature on subacute bacterial endocarditis of the *Streptococcus viridans* type contains few references to the morbid anatomy of the spleen. It is the purpose in the following pages to put on record the results of observations on five cases at the University Hospital and the examination of twenty histologic preparations afforded me by others. Preparations have been received from the following: Dr. McCordoch of St. Louis, six; Dr. Paul of Yale, four; Dr. Bauer of the Pennsylvania Hospital, three; Dr. Black at the Philadelphia General Hospital, three; Dr. Case at the Graduate Hospital, three, and Dr. Reimann at the Lankenau Hospital, one. The records of the cases in Philadelphia were personally reviewed, while those in St. Louis and New Haven were examined and approved as true *viridans* endocarditis by those who sent the material. Only cases in which autopsy had been performed and in which during life or at postmortem examination or both *Streptococcus viridans* was isolated from the blood were accepted. In addition, the cases were reviewed with Horder's criteria in mind. They all showed fever, endocarditis, bacteria at some location and some evidence of embolic dissemination of the organism—petechiae, infarcts or glomerulonephritis.

The principal information concerning the spleen in this disease can be obtained from Clawson's articles.[1] The organ is always enlarged and is greater in size in malignant and in subacute endocarditis than in any other form or phase of this disease. Infarcts form the most conspicuous morbid change, being found in 62 per cent of cases. According to the present studies, these occur most often near the upper pole of the organ and somewhat more frequently on the posterolateral surface. Suppuration is not common, but was noted in five of the twenty-five records included here. The infarcts may participate in the enlargement

* Submitted for publication, April 3, 1930.

* From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

* Read before the American Association of Pathologists and Bacteriologists, New York, April 18, 1930.

1. Clawson, B. J.: An Analysis of Two Hundred and Twenty Cases of Endocarditis, with Special Reference to the Subacute Bacterial Type, Arch. Int. Med. **33**:157, 1924; A Comparison of Acute Rheumatic and Subacute Bacterial Endocarditis, ibid. **37**:66, 1926.

of the organ, and this is apparently confirmed by the figures in the present series of cases. The average recorded weight of all the spleens is 414 Gm. The three without infarcts average 200+ Gm. Nevertheless, as will be seen later in this paper, considerable general hyperplasia and increase of blood can contribute to the enlargement. The cases of this series have been divided, as well as possible from the description, into acute and subacute typical cases of *viridans* endocarditis and acute and subacute cases that are not perfectly typical. It is interesting to note that in the more acute forms the spleen ranges in weight from 250 to 320 Gm., while in the more protracted or more subacute cases the average weights range from 500 to 570 Gm.

Arnett and Clawson have separately shown that phenomena of infection are more potent in enlarging the spleen than is passive congestion. The foregoing statements and those that are to follow agree with their observations.

Concerning the more specific changes in the organ, it may be mentioned that Libman and Aschoff called attention to the prominence of large mononuclears in all tissues, an observation that agrees with the presence of similar cells, especially of the so-called clasmatocyte type, in the blood, probably first noted by Hess.[2] This is also consistent with the studies by Gay on the relation of these large mononuclears in streptococcal disease. They do not, however, loom so large in the microanatomy of the spleen as one might expect from the investigations referred to.

The brief reports of the anatomy of two spleens removed by operation during life in cases of subacute bacterial endocarditis may well be quoted here.

Reimann described the spleen from a case reported by Riesman [3] as follows: "There is endothelial hyperplasia, diffuse around the blood sinuses, just as in acute splenic tumor, e. g. typhoid. The inference is that the patient had a long standing infection of very low virulence or a long standing toxemia."

Escudero and Merlo [4] also reported a splenectomy in a case of this disease. The spleen was large and reddish purple, with various infarcts and an acute perisplenitis at various sites. Cultures were positive for *viridans* organisms.

External to the spleen, but associated with it, have been noted thrombophlebitis and periarterial hemorrhage in the gastric wall. It has been stated repeatedly that pus does not form in *viridans* cases, at least in a manner suggesting it to be the sole effect of the related streptococci.

2. Hess, F. O.: Deutsches Arch. f. klin. Med. **138**:330, 1922.

3. Riesman, David: Chronic Septicemic Endocarditis: The Splenomegaly Treatment by Splenectomy, J. A. M. A. **71**:10, 1918.

4. Escudero and Merlo: Rev. Soc. d. med. int. **1**:361, 1925.

While this may be true, the presence of polymorphonuclears in clumps of various sizes is a frequent observation.

With this brief survey of the pertinent literature, the analysis of the changes found in the series of spleens that I have had may be undertaken. The individuality of the changes peculiar to this disease lies less with the gross than with the minute anatomy, and the latter affords some idea of the character of the lesions that take place in the organ.

GROSS ANATOMIC CHANGES

The spleen in the acute stages or rapidly progressing cases assumed the appearance of an acute tumor of the congestive type—a moderately enlarged, purplish, soft organ, with a smooth, thin capsule, in which there was no capsular inflammation. However, it was the exception to see the spleen at any stage without some perisplenitis, and as the chronicity advanced adhesions became more and more evident. The pulp was usually soft and of a homogeneous purple until late in the disease; then it was firm and dull red. Follicles and trabeculae never seemed overprominent, not even in the two cases that were known to have existed five months. Nor did the larger blood vessels show to the naked eye any definite change. The matters of weight and infarcts have already been discussed.

MICROSCOPIC CHANGES

The twenty-five cases of the present series are divisible into thirteen that seem to fulfil all the requirements of subacute bacterial endocarditis, while the remaining twelve are almost certainly acceptable, yet not so convincing in their history and description. The first group includes those that ran a rapid course and are called acute typical (three), and those in which the history ran to five months and in which the clinicopathologic manifestations were less acute (ten). The spleens do not fall into perfectly corresponding groups, however. In the acute cases and the more acute of the prolonged cases, there was a definite reduction of follicular size, and the lymphocytic pulp was loose and poor in small round cells. The blood content was low, and the blood sinuses and vessels were not conspicuously distended. Reticulo-endothelial cells were not prominent. Pigment was normal in quantity.

In the more advanced cases, there was a striking change in that follicular size increased and germ centers became more evident, reticulo-endothelial cells were vastly increased and much pigment was present.

The first group, then, suggests atrophy or, at least, depressed activity of the tissue, whereas the second group reveals a pronounced activity of all the elements making up the organ.

The twelve cases comprising the acceptable, but not perfect, examples of *viridans* endocarditis are not readily classified with the two

aforementioned groups, but resemble fairly well the second division. The more acute cases of this group are like those of the typical subacute division, but in three of the five in the category there was a complicating mixed infection or unrelated concomitant change. The atypical subacute group did not show splenic changes in discord with any other group, but the description given is not perfectly applicable.

Special Features.—The arterial spindles were not prominent features in the typical case, either acute or subacute, whereas in the aberrant case they might be traced with ease. This does not mean that in the typical case they were eradicated, for they could be found clearly, but there was no prominence of them.

The condition of the germ center commanded attention in five cases by the presence of a coarse coagulum that filled the spaces between a mixture of large mononuclears, adventitial cells and a few polymorphonuclears.

Clasmatocytes (this cellular determination being judged by the irregularity of size with laterally placed nucleus), vacuoles and phagocytic débris were found in any number only in two typical cases. The location was perivascular and perifollicular. Phagocytosis by large nondescript cells was observed in seven instances. In one case, I was inclined to consider the consumed material as red blood cells, but I could not be assured of the correctness of this.

Granulomatoid Collections.—In four cases, cellular accumulations were encountered that challenged attention. They were sometimes perivascular or lateral to a vessel, or they were found without association, except, perhaps, that of being near a follicular border as if near a blood vessel. They consisted of elongated nuclei of connective tissue type, with a few large, round mononuclears and an irregular scattering of polymorphonuclears. The groups were not encapsulated, but around some there was a compression of adjacent cells and fibers that suggested a limitation. The whole area suggested a Bracht-Wächter body of experimental endocarditis and differed from the Aschoff nodule in the absence of large vacuolated cells and the more orderly arrangement of that structure.

A formation possibly akin to this was seen in one case, a case of atypical endocarditis on a background of previous malaria. Scars of a shape and position suggesting removed follicles were found. The center was occupied by fibroblasts and granular débris.

Polymorphonuclear neutrophils were conspicuous in the spleen in subacute bacterial endocarditis in this series. In all stages, typical and slightly atypical, these cells were scattered through the tissue singly and in small groups. Nor were they near infarcts. The position of most of the polymorphonuclears was along cellular groups that corresponded to lymphatic cords, adjacent to blood vessels, near infarcts and in

follicular centers. It cannot be stated that any one of these localities represented a characteristic localization, unless the frequency with which they were formed in germinal centers is worthy of emphasis.

Eosinophils were more numerous in this series of spleens than they were in another series from cases of splenic anemia. Their distribution was not characteristic, but they were perhaps most easily found where the neutrophilic polymorphonuclears occurred.

Disease of the blood vessels of the follicular centers and interlobular septums was not a prominent change. In supposedly normal spleens, hyaline change of media and occasional obliterative endo-angiitis of small vessels are well known. There was no conspicuous increase of these lesions in the spleens in the cases of subacute endocarditis. Only two of this series showed thrombo-angiitis of large veins, and in both cases this was in proximity to infarcts. Curiously enough, the blood vessels in this series of twenty-five were recorded as markedly altered in only nine instances.

The capsular lesions consisted of fibrinocellular exudate that entered the layers of the capsule, as well as accumulated on its exterior. The process seemed most active near trabeculae, but the material does not permit this to be stated as an absolute observation. The cells were chiefly large and small mononuclears, but occasionally a few neutrophils were mixed with them.

SUMMARY

The outstanding features of the spleen in subacute bacterial endocarditis of the *viridans* type are as follows:

The most important gross lesion is the infarct. Practically every case shows perisplenitis whether an infarct exists or not.

In early stages, the organ is not greatly enlarged, but suggests a soft splenic tumor of the congestive type. In the later stages, the organ is definitely enlarged as the result of hyperplasia of its constituent tissue and the inflammatory and hyperplastic changes incident to infarctions.

Hyperplasia of lymphatic elements proper is not a feature of this disease; on the contrary, those tissues are inactive. Hyperplasia of cells of the reticulo-endothelial series is not seen early, but appears more evident in the cases of longer duration.

Marked changes of the linings of blood vessels is not an outstanding peculiarity of this disease. Evidences of destruction of blood are missing in early, but present in older, cases. Degenerations and coagulation within germ centers are frequently observed.

Structures suggesting Bracht-Wächter bodies have been seen several times. Clasmatocytes are occasionally seen, but rarely in any numbers. Polymorphonuclear neutrophils are prominent, as revealed in spleens of this series of cases of bacterial endocarditis. Eosinophils are also frequently seen.

THE EFFECT OF CERTAIN TOXIC SUBSTANCES IN BACTERIAL CULTURES ON THE MOVEMENT OF THE INTESTINES

IV. THE PRODUCTION AND ACTION OF THE TOXIC SUBSTANCES OF BACILLUS DYSENTERIAE (SHIGA-KRUSE) *

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AND
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It has long been known that toxic substances occur in broth cultures of the Shiga-Kruse type of organisms. A short review of the literature is found in the work of Olitsky and Kligler [1] and an extensive study of the "toxinaemia and bacteriaemia dysenterica" was published by Posselt.[2]

In most of the published experiments, signs of intoxication were observed in the rabbit and described at the end of twenty-four hours following the injection of the toxic materials. At this time, general weakness, paralysis of the extremities and diarrhea were fairly well established. However, knowledge is limited concerning phenomena occurring soon after the injection of the toxic filtrates and the effect of the latter on the movement of the intestines in the intact animal.

REVIEW OF THE LITERATURE

Lucchini [3] studied the action of the toxic products on an isolated strip of intestine and found that small and moderate doses of the toxic substances produced an increase of tone and of rhythmic longitudinal movements, while larger doses arrested all movements. Similar results were obtained when the products were heated at 75 C. for one hour. Tadokoro and Suga [4] perfused isolated rabbit intestines with a potent filtrate and observed increased peristalsis with small doses and paralysis with larger doses. As this action was exhibited after heating of the filtrate at 85 C. for one hour, the authors concluded that it was due to other factors than

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* From the Institute of Pathology, Western Reserve University.
* Aided by a grant from the Therapeutic Research Committee of the American Medical Association.

1. Olitsky, P. K., and Kligler, T. J.: J. Exper. Med. **31**:19, 1920.
2. Posselt, A.: Ergebn. d. allg. Path. u. path. Anat. **22**:360, 1922.
3. Lucchini: Igiene mod. **6**:301, 1923; quoted by Posselt.
4. Tadokoro, R., and Suga, K.: Keio Igaku, 1925, vol. 5, no. 17; abstr., Japan M. World **6**:67, 1926.

to the toxin itself. This conclusion applies also to the work of Lucchini.[3] Creazzo [5] pointed out that the toxic effect may be on the plexus of Auerbach. The clinical symptoms in dysentery, it is suggested by Tadakoro and Suga, may be due to the stimulation of the accessory sympathetic nerves of the larger intestine instead of to a nonspecific reaction arising from a pathologic change in the mucous membrane.

Loeper [6] recorded degenerative changes of nerve fibers and of cells of the plexuses in dysentery of man, and these changes occurred particularly in the region of Peyer's patches. Lorentzen [7] found early in the disease changes in the ganglion cells of the intestine (Meissner's plexus) in the form of vacuolization, poor staining and necrosis. The process may extend to the cells of Auerbach's plexus. These changes one must take into consideration when dealing with intestinal motility in this disease.

EXPERIMENTS

The present report concerns a systematic study of the effect of the filtrates of stock and newly isolated cultures of various ages on the movement of the small intestine in the intact rabbit and in the laparotomized rabbit, by the use of both the "pouch technic," as described by Ecker and Rademaekers,[8] and the new method of Ecker and Biskind,[9] which eliminates ethyl carbamate (urethane) anesthesia.

Method.—Strains 51, 52, 858, 859, 860 and 861 employed in these experiments were obtained through Dr. G. H. Weaver from the American collection of culture types and were all recently isolated when employed. One strain was secured through Dr. W. B. Wherry and one strain (Deli) through Prof. E. P. Snyders. Strain 731 (A-2440) was supplied by Dr. C. TenBroeck, of Peiping, China. The mediums employed were a 2 per cent Witte peptone veal infusion broth, with a p_H of from 7.2 to 7.4, and the egg albumin broth of Olitsky and Kligler. The organisms were grown at 37 C. for various periods of time, and the cultures were passed through Berkefeld N candles and the filtrates tested for sterility. From 1.5 to 4 cc. of the filtrates was injected into the marginal ear vein of the rabbit. While the effect of a filtrate on the motility of the laparotomized rabbit's intestine was being studied, an intact control rabbit was given the same dose in order to determine the potency of the filtrate. Additional animals, intact and laparotomized, received the sterile noninoculated broth, with no other effects than those described by us elsewhere (Ecker and Rademaekers [8]). The filtrates were heated at 60 C. for thirty minutes and at 100 C. for five minutes to determine the effect of heat on their potency.

5. Creazzo: Arch. per le sc. med. **35**:361, 1911.

6. Loeper, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **43**:196, 1919; Progrès méd. **34**:129, 1919.

7. Lorentzen, W.: Virchows Arch. f. path. Anat. **240**:184, 1922.

8. Ecker, E. E., and Rademaekers, A.: J. Exper. Med. **43**:785, 1926.

9. Ecker, E. E., and Biskind, M. S.: The Effect of Certain Toxic Substances in Bacterial Cultures on the Intestinal Movement, II, Arch. Path. **7**:204, 1929.

The pouch method of Sollmann for the study of the rhythmic longitudinal muscle movements, as here employed, has been described elsewhere.[8] The operations were all performed under anesthesia produced by ethylcarbonate (2 Gm. per kilogram), which was administered through a stomach tube. Additional studies were made by the new method of Ecker and Biskind.[9] Prior to the experiment, all food was withheld for twenty-four hours.

The Production of Toxic Filtrates and Their Effect on the Rabbit.—Table 1 summarizes the observations on the toxicity of filtrates of cultures of various ages. The Wherry strain was grown in 2 per cent Witte peptone veal infusion broth for from twenty-four to one hundred

TABLE 1.—*Yield of Toxic Substances in Plain Broth Cultures of Various Ages (Strain Wherry)*

Rabbit's Weight, Gm.	Age of Culture When Filtered, Hours	Amount of Filtrate Injected, Cc.	Hour of Injection	Results
1,700	24	1.5	10:15	At 11:12, respiration rate is variable; at 11:16, rabbit urinates and flattens; at 11:16, hind legs are weak; at 11:40, rabbit recovers
1,970	24	3	1:15	At 1:50, respiration rate is irregular; at 2:10, rabbit urinates, and hind legs are weak; at 2:15, rabbit flattens; at 2:20, it recovers
1,450	48	1.5	9:50	At 10:48, rabbit urinates and defecates; at 10:55, there is marked defecation; at 11, mild paresis of hind legs is present and respiration is irregular; at 11:15, rabbit recovers; next day, in afternoon, it shows paresis of legs, is very weak, but recovers later
1,508	72	1.5	9:35	At 10:15, rabbit urinates and has paresis of hind legs; at 10:20, it flattens and passes soft stool; at 10:35, it passes more soft stool; at 10:45, it has paresis of front legs, prostration and spasms; at 10:50, it has convulsions; at 10:55, it is dead
1,850	120	1.5	10:53	At 11:30, rabbit shows incoordination; at 11:45, its respiration is labored and it is nervous; at 11:50, it flattens; at 11:55, respiration is extremely labored and irregular; rabbit recovers
2,300	..	Sterile broth filtrate 3, control	10:48	No reaction

and twenty hours, and the cultures were then filtered through a Berkefeld N candle and tested for sterility.

From table 1 it is seen that a definite reaction occurred within one hour following the intravenous injection of the filtrate of the culture grown for twenty-four hours. The animal inoculated with a filtrate of a three day old culture died in one hour and twenty minutes, thus demonstrating the occurrence of an early acute reaction. This is comparable to the more pronounced reaction that we have reported as obtained by the use of young cultures of the paratyphoid group.

The majority of the animals survived the immediate reaction. Rabbit 6, inoculated with the filtrate of a forty-eight hour old culture, showed typical reactions of the nervous system on the day following the injection, but no diarrhea. In the case of this particular strain, then, a minimum of forty-eight hours of incubation in this broth was necessary to

obtain a markedly toxic filtrate. Since diarrhea was not common in these animals, we decided to use the egg albumin broth of Olitsky and Kligler and also other strains of the Shiga-Kruse organisms.

Table 2 gives a résumé of these observations. It is evident from this table that the organisms grown in egg albumin broth produced potent filtrates. Early and marked reactions occurred in all the animals receiving injections. Intestinal reactions (diarrhea) were present in eight of the eleven rabbits receiving the injections. These occurred in from forty-five to eighty minutes following the injections of the toxin. Nervous symptoms were noted both early and late in all but one of the animals. Two of the rabbits receiving injections survived, and seven died within twenty-four hours. Two others died on the third day following the administration of the filtrate. Two died within four hours after the injection of the filtrate. Of the six strains of *B. dysenteriae* (Shiga-Kruse) employed, not one failed to produce a potent filtrate. Severe diarrhea and paralysis were also produced in animals that received saline washings of the Wherry strain grown for twenty-four hours at 37 C. in Kolle flasks on plain agar. The washings thus secured were incubated at 37 C. for three days prior to filtration.

Resistance of the Toxic Filtrates to Heat.—In a series of experiments (table 3), we determined the effect of heat on the potency of the filtrates of three and five day old growths of two strains in plain broth and in egg albumin broth.

Heating of the filtrates of three and five day old growths of the Wherry strain at 60 C. for thirty minutes and at 100 C. for five minutes left the filtrates practically unaltered in potency. The early reactions were similar to the reactions obtained following the injection of the nonheated filtrates. Filtrates of the Deli strain grown for three and for five days in veal infusion broth and heated at 100 C. for thirty and for sixty minutes showed a definite decrease in potency. On injection of a filtrate of a three day old culture of this strain heated at 100 C. for one hour, the rabbits appeared weak and discharged a moderate amount of soft feces. One of the two animals showed a moderate paresis of the hind legs. Heating of this filtrate at 100 C. for thirty minutes was insufficient to weaken the filtrate to the extent of the one heated at 100 C. for one hour. The reactions were marked, and one rabbit was found paralyzed, with a marked fecal discharge on the day following the injection. The reactions noted following the injection of filtrates of this strain were of the nervous type. The filtrate of a five day old culture of this strain was greatly weakened following heating at 100 C. for one hour. The control animal died in three days with all the typical symptoms.

TABLE 2.—*Yield of Toxic Substances in Egg Albumin Broth by Several Strains of B. Dysenteriae (Shiga-Kruse)*

Rabbit's Weight, Gm.	Number or Name of Culture	Age, Hours	Amount of Filtrate Injected, Cc.	Hour of Injection	Results
2,190	Cinci	120	1.5	3:00	At 4, rabbit is very excitable; at 4:15, it has convulsions; at 4:25, it has more convulsions and dies
1,950	Cinci	120 (new culture)	1.5	1:45	At 2:27, rabbit urinates; at 2:30, it flattens, has diarrhea and is nervous; at 2:45, shows marked diarrhea and at 3:10, tremors; at 5:30, it shows paresis of hind legs and lies on side; at 5:40, it has convulsions; at 5:45, it is dead
1,420	731	120	1.5	11:33	At 12, rabbit has marked diarrhea and at 12:20, still more diarrhea; at 12:24, it urinates; at 12:30, it has paresis of hind legs; it remains sick all afternoon and evening; next day it is very sick, lies on side and is paralyzed; it survives
2,130	731	120	2.0	11:00	At 12, rabbit has diarrhea with urination and cramps; at 12:10, it passes liquid feces; at 12:12, more liquid feces and at 12:27, a mucous discharge; it dies during the night
1,605	731	72	1.5	10:25	At 10.45, rabbit defecates; at 10:47, it urinates, is prostrated, swings from side to side; at 11:15, it passes soft fecal balls; at 11:35, there is marked defecation of soft stool; two days later, rabbit shows complete paralysis of front and hind legs and diarrhea and dies on the third day following injection
1,700	51	120	1.5	1:55	At 2:40, rabbit urinates; at 3, it defecates; at 3:05, it has cramps with defecation; at 3:15, it flattens; at 3:20, the hind legs are weak; 48 hours later, it has paralysis of front legs, soft feces, marked diarrhea and beginning paralysis of hind legs; on third day after injection, rabbit dies
1,900	51	120	1.5	10:50	At 11:45, rabbit urinates; at 11:46, it passes soft feces; at 11:52, it has diarrhea; at 12, it has a mucous discharge and is weak; next day it is very weak and dies
1,550	860	120	1.5	11:45	At 12:15, rabbit defecates and urinates; at 12:30, it flattens, and respiration is rapid; at 12:35, it passes soft feces; at 12:37, it has diarrhea and paresis of hind legs; at 12:45 and 12:55, there is more diarrhea. Rabbit appeared recovered in the afternoon
1,550	860	120	1.5	11:05	At 11:55, rabbit urinates; at 12:10, it is nervous and at 12:12, hyperpneic; at 12:20, it defecates; at 12:30, it is very excitable, showing twitchings of muscles, and it remains so all afternoon; at night, rabbit dies
1,500	859	120	1.5	11:00	At 11:48, rabbit defecates; respiration is labored; at 12, rabbit flattens and is nervous; at 12:35, it is weak in the hind legs and excitable; at night, it dies
1,900	861	120	1.5	11:20	At 11:40, rabbit urinates; at 12, it appears spastic with cramps; at 12:07, respiration is labored; at 12:08, rabbit is restless; at 12:13, it shows air hunger; at 12:15, it is extremely excitable; at 12:30 it urinates and at 2, defecates; during night, it has diarrhea and is dead the next morning

Effect of the Filtrates on Intestinal Movements.—Eleven experiments were made with the Sollmann pouch technic in order to observe directly the reactions of the small intestines to the filtrates. The dosages used varied from 1.5 to 4 cc. of filtrates of three to six day old cultures of

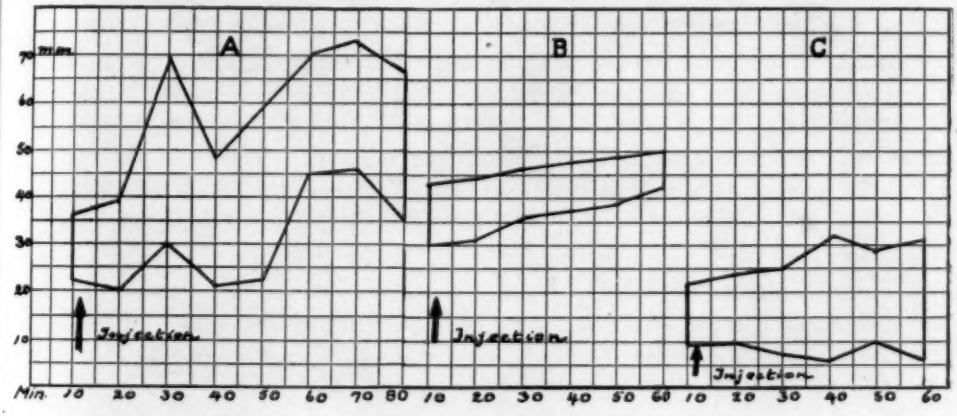
TABLE 3.—*Effect of Temperature on Potency of Toxic Filtrates of the Wherry Strain*

Rabbit's Weight, Gm.	Age of Culture When Filtered, Hours	Exposure to Heat, Degrees C.	Time of Exposure, Minutes	Amount of Filtrate Injected, Cc.	Hour of Injection	Results
			Plain Broth Cultures			
1,508	72	0	0	1.5	9:35	At 10:15, rabbit urinates and has paresis of hind legs; at 10:20, it flattens and passes soft stool; at 10:45, it it passes more soft stool; at 10:45, it has paresis of front legs, prostration, spasms, and at 10:50 convulsions; at 10:55, it dies
1,550	72	60	30	1.5	3:05	At 3:45, respiration deepens; at 3:55, animal urinates; at 4, it has marked diarrhea; at 4:45, it is very weak and lies on side; at 5:20, it is slightly recovered; next day, it is very weak and at night has considerable diarrhea, and dies
1,850	72	100	5	1.5	3:03	At 4:03, respiration is increased from 120 to 200+; at 4:45, animal is prostrated and has paresis of hind legs; at 5:15, it dies in convulsions
			Egg Albumin Broth			
1,950	120	..	..	1.5	1:45	At 2:27, rabbit urinates; at 2:30, it flattens, has diarrhea and is nervous; at 2:45, it has marked diarrhea; at 3:10, tremors; at 5:30, paresis of hind legs, lying on side; at 5:40, convulsions; at 5:45, rabbit is dead
2,300	120	..	..	1.5	10:00	At 10:10, rabbit urinates; at 10:35, respiration is labored; at 10:40, rabbit urinates; at 10:45, respiration is irregular; at 11:05, there is marked defecation (soft feces); rabbit presents no further symptoms and recovers
1,720	120	60	30	1.5	2:20	At 2:50, respiration is irregular; at 3:05, rabbit is extremely nervous and has slight convulsions; at 3:30, it is prostrated; at 3:33, it shows air hunger and convulsions; at 3:35, it is dead
1,900	120	60	30	1.5	10:15	At 10:25, rabbit urinates; at 10:40, respiration is labored; at 10:45, animal is extremely nervous and dyspneic, and defecates; at 10:55, there is more defecation, and at 1:15, intermittent convulsions; at 1:45, it is dead
2,150	120	100	5	1.5	10:27	At 11:25, rabbit flattens; at 11:30, it urinates and shows copious discharge of fecal balls; at 11:32, it has diarrhea and is very nervous. In ten days it is dead. It remained weak and sick throughout the ten days

strains 731, 941, 858, 860, 861 and Wherry. All these filtrates were highly toxic for the control rabbits. Three types of reactions were noted. In four of the eleven experiments recorded here in composite form, an increase of tone of the longitudinal muscles with an increase of the amplitude of contractions was observed (graph *A*), and in five experiments similarly recorded, an increase of tone with a decrease in the amplitude of contractions (graph *B*). In two experiments, a rise

in amplitude with no change in tone was found (graph *C*). The graphs demonstrate the type of reactions observed.

A control series of intact rabbits received the same filtrates at the same time as the laparotomized rabbits. When the reactions of the intact and of the laparotomized animals were compared, it was found that filtrates which caused diarrhea in the normal rabbits usually caused a rise of tone of the intestines in the laparotomized animals. Filtrates that caused severe reactions of the nervous system caused the most marked rises of tone, accompanied, however, by a decrease of amplitude of contractions. Usually the diarrhea observed in the intact animal preceded the changes noted in the laparotomized animals. This may be accounted for by the inhibiting influence of the urethane anesthesia.



Graphs showing changes of tone and of the amplitude of contraction of the longitudinal muscles following injection of filtrates of cultures of B. dysenteriae (Shiga-Kruse): *A*, an increase of tone with an increase of the amplitude of contractions; *B*, an increase of tone with a decrease of the amplitude of contractions, and *C*, an increase of the amplitude of contractions with no change in tone.

In four experiments, an increase of tone and of amplitude of contractions in the laparotomized animals was observed, while the control animals had severe diarrhea.

Strain 731 produced a rise of tone of the longitudinal musculature, with a variable effect on the amplitude of contractions. The Wherry strain caused a rise of tone, accompanied by a decrease of amplitude of contractions. These facts demonstrate the variability of the reactions that may be obtained with filtrates of different strains of this organism. Animals also differ in susceptibility. Certain strains (861 and Deli) produced severe neurotoxic effects.

Variation in Susceptibility of Animals.—To meet the question of individual variability of the animals, 4 cc. of a filtrate of a one hundred

and forty-four hour culture of strain 731 was injected into each of four animals. The resulting reactions were strikingly similar, namely, a transitory increase in the rate of respiration of the animal, then a slowing with increased depth, urination at the end of one hour, diarrhea and death within from twenty-four to forty-eight hours. The animals did not show symptoms of involvement of the nervous system.

Relation of Age of Culture Filtrates to Type of Reactions Produced.—Repeated tests showed that the age of the culture was of some importance with regard to both symptoms and ultimate prognosis. Filtrates of twenty-four hour old cultures usually produced mild nervous symptoms with no diarrhea. Filtrates of forty-eight hour old cultures caused increased nervous symptoms with defecation, but not diarrhea. No deaths followed injections of filtrates of cultures from twenty-four to forty-eight hours old. Filtrates of seventy-two hour old cultures produced the most consistent symptoms of dysentery, namely: urination, paresis and paralysis of the hind and front legs, diarrhea, local spasms and general convulsions and death. The reactions began uniformly one hour after the injections of the filtrates.

A filtrate of a fourteen day old culture produced marked nervous symptoms, namely: dyspnea, prostration, paresis, paralysis and death in less than twenty-four hours. Here no diarrhea was noted.

Observations on Intestines under Oil According to the Method of Ecker and Biskind.—In a series of four experiments performed by the method of Ecker and Biskind,[9] the filtrates of three strains, nos. 986, 731 and 52, were employed. The ages of the cultures were 4, 5, 6 and 30 days. The normal control animals showed prostration, defecation and neurotoxic reactions. The laparotomized animals showed an increase of contractions in the longitudinal muscles of the upper intestines and an increased activity of the lower colon, with marked propulsion. The lower colon appeared to be more active than usual. The most marked effect was obtained when the filtrate of a month old culture of strain 731 was injected. In this case, the tone of the cecum was markedly increased, and rolling movements of the haustra of the colon were noted. In general, the reactions were mild, and the results compared favorably with those obtained by the older method.

SUMMARY

Although toxic manifestations were noted in rabbits that received filtrates of twenty-four to forty-eight hour old cultures by vein, the most marked reactions occurred in those animals that received filtrates of seventy-two to one hundred and twenty hour cultures. This was true of both the filtrates of cultures grown on 2 per cent Witte peptone veal infusion broth and those of cultures grown on the egg albumin

medium of Olitsky and Kligler. Diarrhea was more common in these animals than in the animals inoculated with the filtrates of younger cultures.

Sardjito [10] also noted that the formation of these toxic substances did not begin until after the third day of incubation. Okell and Blake [11] observed that viable cells appear in their greatest number in twelve hours, and that filtrates usually attain a maximum toxicity when a p_H of from 8.6 to 8.8 has been reached. During the period of increasing toxicity, autolytic changes (as gaged by microscopic observations of the bacilli) become more and more marked. They described two stages in the production of these toxic substances: "(1) a growth (or endotoxic) phase lasting 12 to 18 hours when all the toxin is produced as endotoxin within the bodies" and "(2) an autolytic (or exotoxic) phase during which autolytic processes gradually disintegrate the bacilli and release the endotoxin into the circumambient medium (filtrate)."

As stated before, powerful toxic substances did not occur in our very young cultures. We agree with Kanai,[12] Przesmycki [13] and Robertson [14] that it is at present difficult to split an exotoxin and an endotoxin in the sense of Pfeiffer. However, Olitsky and Kligler stated that such a differentiation can be made. It is also possible that the type of toxic elements produced in vivo may greatly differ from that produced in vitro (Lüdke [15]), and that different products are elaborated during the growth of the organism.

Of thirty-eight animals into which the filtrates of various strains of the organism were injected, twenty-eight died. Thirty-two animals showed involvement of the central nervous system (medulla and cord), and twenty-nine showed diarrhea of varying degrees of severity. Kanai [12] showed that the toxin of this organism affects principally the central nervous system and at the same time acts on the capillary circulation generally, with the production of hyperemia and hemorrhages in the various viscera. Olitsky and Kligler [1] believed that the so-called exotoxin operates on the central nervous system and that the endotoxin affects the gastro-intestinal tract. At present, it is doubtful that divisions into distinct fractions can be accomplished, in view of the complexity of the mediums employed for the production of these substances, etc. The reactions observed in the laparotomized animals were not marked. They appeared variable, a rise of tone with an increase of amplitude of contractions and also with a decrease of amplitude

10. Sardjito, M.: Geneesk. tijdschr. v. Ned. Indië **66**:337, 1926.
11. Okell, C. C., and Blake, A. V.: J. Path. & Bact. **33**:57, 1930.
12. Kanai, S.: Brit. J. Exper. Path. **3**:158, 1922.
13. Przesmycki, F.: Med. dosw. i. spol. **5**:18, 1925.
14. Robertson, R. C.: Brit. M. J. **2**:729, 1922.
15. Lüdke, H.: Deutsche med. Wchnschr. **50**:1569, 1924.

of contractions of the longitudinal musculature being seen. Furthermore, a simple increase of the amplitude of contractions without a rise of tone was also recorded. Direct observations under oil gave the impression of an increased activity of the lower colon following the injection of the filtrates. At no time, however, was a peristaltic rush observed. It seems justifiable to assume that the filtrates, owing to their variable neurotoxic qualities, affect the vagus in a greater or less degree, or that the preexisting tone of the intestines accounts for the reactions seen. Robertson [14] believed that the neurotoxin has a secondary action on the intestine, through the initial inhibition of peristalsis prior to the passing of mucous or desquamated epithelial débris. Additional studies are required to elucidate further the mechanism in question.

General Review

JUVENILE ARTERIOSCLEROSIS *

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Arteriosclerosis occurs in children much more frequently than is commonly believed. Even in infancy may be found not only the early manifestations of the disease, but occasionally stages so far advanced as to present extensive calcification. A careful study of the disease in the early years of life is especially valuable from an etiologic standpoint, since in such cases many of the complicating factors of adult life are absent (Dickinson).

The term "arteriosclerosis" was first used by Lobstein in 1834 (cited by Collins). Later, Jores limited its use to those conditions in which there was definite fatty degeneration in the intima. Marchand used the term in a much broader sense, including all changes in the coats of arteries that lead to a thickening of the wall. Klotz, in 1915, employed the term in a generic sense, not to define any particular disease, but to denote a process induced by a variety of factors the end-result of which is a hardening of the arteries. Later modifications of the definition may be found in the writings of Osler (1918) and Evans (1923).

In the present study, an attempt has been made to include all cases in which there was definite thickening, hardening or scarring of the arterial walls resulting from degenerative and nonspecific inflammatory processes, thus excluding all cases in which the disease was frankly syphilitic, rheumatic or immediately pyogenic in origin, and also omitting those in which the lesions were the results of operative procedures or traumatic accidents. Furthermore, this study does not include periarteritis nodosa or the lesions known as the thrombo-angiitis obliterans of Leo Buerger.

The lesion of particular interest in this review is the so-called simple arteriosclerosis, of undetermined etiology, commonly believed to begin in the intima with lipoid degeneration (Saltykow, 1926), often followed by calcification. The process may or may not be accompanied by a definite inflammatory cellular reaction; it may be focal or diffuse; it may be limited to the intima or may involve all coats. Sometimes it involves

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only the aorta and large branches; at other times, it affects the visceral arterioles, and again it may be most manifest in the media of peripheral arteries.

Although the term "juvenile" has been used by certain authors to refer to a particular type of arteriosclerosis occurring at any age, and by others to include any type occurring before the third or fourth decade, this review will be concerned only with cases occurring before the twentieth year of life.

EARLY PERIOD OF INVESTIGATION, BEFORE 1872

The first mention of this condition in children was by Hodgson in 1815 (cited by Fremont-Smith), who described the temporal artery of a 15 months old infant as being "converted into a complete tube of calcareous matter." In 1826, Martin stated that he had observed changes in the aorta after the first year, but had never seen disease of the middle-sized branches before the twentieth year (cited by Fremont-Smith). Three years later, Andral noted calcified plaques in the aorta of an 8 year old girl, but thought that they occurred in the media. He said, however, that the internal coat was often "detached from its connections by the calcium concretions" and later stated "the bony material is simply deposited between the internal and middle coats." He even suggested that "the bony matter may originate in the atheromatous material," which he frankly recognized as being located in the intima. He further said, "All we know of the formation of these ossifications is that they are the result of a derangement in the natural processes of secretion and nutrition. . . . It is a general and constitutional derangement associated with too free use of animal diet." A hundred years have passed since this was written, but the etiology of arteriosclerosis is still obscure.

During the twenty-five years following Andral's publications, little is found in the literature concerning this condition in children. In 1855, Rokitansky's "Pathological Anatomy" was published, in which is the suggestion that the atheromatous plaques may be derived from the blood. The author observed that the "deposition is generally thickest directly over the division of a trunk, or at the bifurcation of a vessel." He strictly differentiated this process, which he considered degenerative, from a true inflammation. In regard to age, he recognized the occasional absence of the disease in old persons, as well as its occurrence in childhood, and believed that when it occurs prior to the age of 20 years it is usually a local disease, depending on congenital or early acquired anomalies of the blood vessels or heart. He considered the disease to be caused by a peculiar condition of the arterial blood, apparently antagonistic to tuberculosis and associated with an excess of fat and cholesterol. He

was one of the first to direct attention to the rarity and peculiarities of this condition in the veins.

During the following decade so little was heard of this condition in children that Lebert in 1867 wrote: "In youth atheroma is very rare; it begins usually after the 40th year of life." A similar opinion was expressed three years later by Steiner and Neuretter, and during the same year Holmes wrote, "The gouty, the aged, the rheumatic, the persons whose tissues are embued with fat, and those whose excretory organs fail to purify the circulating blood, are more prone to this disease than the phthisical, the cancerous, or the young." In 1872, De Mussey, in a thorough review of all phases of hardening of the arteries, stated that he had seen the condition in persons "as young as 17 years."

MIDDLE PERIOD, 1872-1900

The year 1872 marked a milestone in the study of arteriosclerosis, for it was in that year that Sir William Gull and Henry G. Sutton published their often-to-be-quoted paper, "Chronic Bright's Disease." Besides stressing the importance of arteriolar changes in this condition, they reviewed 336 cases that had come to autopsy, one of these being that of a 9 year old child. Not only did this article and others by the same authors arouse widespread discussion and dissension, but workers everywhere began to note more carefully changes in the vascular system in all kinds of cases and at all ages. Chronic nephritis became of especial interest and was studied not only in adults but also in children. So, in 1874, Barlow reported the case of a young child with vascular changes associated with contracted kidney. The following year a case of aortic disease unassociated with renal lesions was observed in France (Moutard-Martin).

In 1881, the results of a decade of careful observation and study began to appear in the literature. Tyson recognized an association between chronic nephritis and diabetes and also noted changes in the vascular system in both diseases. Phänomenow reported a case of abdominal aneurysm in a newly born baby, probably atherosclerotic in origin. But of most importance to this study was the appearance in that year of Dickinson's masterly "Treatise on Albuminuria," in which he cited many cases of undoubted arteriosclerosis associated with renal disease in children. He also recognized an increased arterial tension in such cases and described muscular hypertrophy in heart and blood vessels as following an "overexertion of the heart and arteries" attempting to drive blood through "vessels which seem to be overfull." But he did not believe, as did some of his successors, that this muscular hypertrophy constituted the sum and substance of arteriosclerosis, but stated, "The

arteries, besides mere muscular thickening, undergo degenerative and pseudo-inflammatory changes, palpable atheroma, nuclear and muscular degeneration and marked thickening of the fibroid sheath." He also appreciated the tendency for hemorrhages to occur in the type of arteriosclerosis associated with renal disease. He said, "In granular degeneration of the kidney in children the vascular change is extreme and characteristic, the hypertrophy of the left ventricle great, and cerebral hemorrhage an occasional termination." He believed that the "cardiovascular thickening is a direct consequence of simple renal inflammation . . . and is directly produced by it."

In 1883, the influence of Gull and Sutton was manifested in Germany in Filatoff's diagnosis of "arteriolar capillary fibrosis" with contracted kidneys and atheromatous arterial degeneration in a 12 year old boy. The following year, Thoma expressed his views on the influence of mechanical factors in causing the closure of arteries in amputation stumps. These observations were followed by years of the most painstaking experimentation on his part to prove the mechanical origin of sclerotic plaques in blood vessels. Thoma (1886, 1911 and 1922) believed that thickening of the intima by connective tissue followed slowing of the blood stream. Although other workers (Ophüls, 1906) repeated his experiments, with different results, causing much of his work to stand discredited today, nevertheless Thoma helped to emphasize the need for careful experimentation in the field of arteriosclerosis and thus made a valuable contribution to this subject.

During the next five years, reports of seven more cases appeared in the literature (table 1). In 1887, Sanne published a review of the subject, "Aneurysm in Children." He concluded, "The pathology is the same as in adults. To ascertain the underlying cause one must study the heredity and past history of the patient."

In Keating's "Encyclopedia of Diseases of Children," one again finds emphasis placed on the rarity of atheroma in youth. The author stated that when it does occur it is found usually in the blood vessels of the brain and in the aorta. During the same year, Girode reported two cases of marked arteriosclerosis in young boys addicted to alcohol.

In 1893, Basch published a series of articles on latent arteriosclerosis, but was more interested in the changes occurring during the third and fourth decades of life than in those of childhood. Other writers, however, have recognized similar latent changes in young persons.

Döhle's article in 1895 on syphilitic disease of the aorta, followed in 1897 by Dmitrijeff's description of the changes in elastic tissue in arteriosclerosis, emphasized the growing opinion that syphilis produces a definite pathologic picture that can usually be differentiated from other

types of arterial disease. Henceforth, fewer authors gave syphilis as the probable cause of atheromatous lesions (Allbutt, 1899).

Seitz (1896) expressed the opinion that arterial changes in the young were not so rare as had commonly been believed to that time. Besides collecting a considerable number of cases from the literature, he reviewed in detail 3 of his own cases, and also stated that among 148 cadavers examined in the Munich Pathological Institute he found characteristic changes in the blood vessels of 17 persons that were between 10 and 29 years of age. Also, he had encountered numerous cases clinically. He believed that in his cases he could fairly well rule out presenile changes, alcoholism, muscle strain, syphilis and intestinal intoxication, but thought heredity might be an important etiologic factor. This factor was again emphasized in 1899 by Brill, especially in cases associated with chronic nephritis. During the same year, Durante described a case of aortic disease developing in utero. The hereditary factor was emphasized as recently as 1925 (Mortensen) and 1926 (Leopold).

In spite of all these cases in the literature, Baginsky in 1899 wrote: "Aneurysm and chronic endarteritis are very rare in childhood."

RECENT PERIOD, 1900-1930

During the first five years of the period from 1900 to 1930 reports of cases and observations appeared in the literature from many sources. Probably there were two chief reasons for this: first, the developing interest in infection as an etiologic factor in arterial disease, inducing more careful examinations of the vascular system in deaths from the infectious diseases of childhood (Stengel; Simnitzky; Seitz, 1901; Buchta; Jordan; Gilbert and Lion; Torhorst, and Thayer); second, the reopening of the subject of chronic nephritis in children (Guthrie, cited by Greene; Baines; Milligan, cited by Greene; Hirsh; Anderson; Democh). It was also at this time that Bryant and White described their interesting case of extensive vascular sclerosis with calcification in a 6 months old baby in whom the only obvious etiologic factor was extreme phimosis, with urinary obstruction.

Parallel with the study of arteriosclerosis in children, intensive work was done during this period on the general subject of vascular disease, much of which was in the experimental field (Jores, Matusewicz and others).

During 1904, a comprehensive series of reports appeared presenting the data for and against the various agents suspected of causing arteriosclerosis (Cabot, Stengel, Thayer, Billings, Dock). During the same year, a similar discussion was carried forward in the Congress für innere Medizin in Germany. Here Romberg mentioned the frequency with which he had observed arteriosclerotic changes in young persons, many

of whom did not have chronic nephritis. Schott had frequently seen the condition in youth, and believed that in such cases the disease might remain stationary for a long time, or was capable of flaring up and progressing rapidly.

The following decade (1905 to 1914) added many articles summarizing, commenting on, and occasionally contributing more data to, the accumulated evidence that infection plays an important etiologic rôle in arteriosclerosis. Wiesner and Wiesel found changes in the coronary arteries in young persons with verrucous endocarditis, osteomyelitis and other acute infections. Saltykow (1908) and Manouelian produced atherosclerosis in animals by repeated injections of staphylococci. Lubarsch found atherosclerosis in young dogs dying of acute infections.

Frothingham studied the relationship between infectious diseases and arterial lesions in man. He found vascular lesions in fifty-six persons under 25 years of age, and in nearly all persons over that age who suffered from infectious diseases. In all of his patients over 1½ years old dying of tuberculosis, typhoid fever, glanders or pneumonia there were intimal changes in the aorta. Frothingham believed that in patients who recover from acute infections, the vascular changes may also disappear, except those in which actual necrosis has occurred. In such cases, healing is by the formation of connective tissue and the scar is permanent. The lesions that heal without scarring consist of fatty droplets in the tissue cells. In 1915, Klotz expressed a similar opinion. In a later article (1913), Frothingham discussed all the various factors believed to be of etiologic importance in arteriosclerosis and concluded: "It is certain, therefore, that infections may cause localized arterial lesions but the evidence in regard to their causing diffuse arterial disease is lacking. . . . As to the most likely causative agents in production of arteriosclerosis, the most evidence at present is in favor of retained metabolic products through faulty elimination, and acute infections."

During 1913, Hirsch in Berlin emphasized the hereditary factor, especially in juvenile cases. He also differentiated arteriosclerosis from "angio spasm" and "nervous heart," which often present similar symptoms.

During this period, the effect of epinephrine and high blood pressure on the cardiovascular system was studied extensively (Josue, Harvey). By injecting epinephrine hydrochloride, Fischer produced aneurysms, and Ziegler, necrosis and calcification of the media with compensatory intimal thickening. By the use of amyl nitrite, Braun counteracted the effect of epinephrine in raising blood pressure, and found the same type of pathologic lesions as when epinephrine was used alone; Boveri modified the severity of the lesions following the administration of epineph-

rine by the use of iodized sesame oil 40 per cent. Rickett obtained lesions in the media by using mechanical means of raising blood pressure. In 1909, Harvey pointed out that the lesions of experimental arteriosclerosis were a medial degeneration of the Mönkeburg type, while Sumikawa showed that sclerosis of the human aorta and large vessels begins in the intima.

The trend of thought in relation to juvenile arteriosclerosis was decidedly affected by the experimental work of this period. Ferenczi looked on vascular lesions in childhood as a sign of exhaustion, which, under ordinary conditions, occurs only in old age, but occasionally, as a result of hard physical labor, toxins, high blood pressure, etc., the "resistance capacity" of the vascular system may be exhausted early in life. A similar opinion was expressed by Romberg, who called it a "wearing-out process" and recognized its frequent occurrence in young persons.

In 1912, Klotz wrote, "If we wish to gain a true insight into the complex question of arteriosclerosis we must attempt to follow the lesion from its earliest beginning." He mentioned grossly visible superficial fatty streaks in the blood vessels of persons from 1 to 73 years old (the majority being between the ages of 20 and 30 years). Changes were found in the aorta more frequently than in any other vessel. The fatty streaks were rarely seen before the tenth year of life; he mentioned only four cases all of which followed scarlet fever. Furthermore, Klotz differentiated fatty intimal streaking from the nodular pearly thickening seen at orifices of blood vessels and in the walls of peripheral arteries. Fatty streaks were particularly associated with infectious diseases, such as typhoid fever, pneumonia and acute osteomyelitis. In only two of thirty-five cases of typhoid fever in which blood vessels were examined for yellow streaking, was it absent. Jores was the first to show that these yellow streaks in the aorta in young persons were the beginnings of arteriosclerosis. Torhorst did not see much relationship between the two conditions. Zinserling studied the polarizing effect of these fatty particles in the young and found it to be the same as in older people. They occurred with great frequency in young persons (from 16 to 17 years old) dying of typhoid fever, also in children from 6 to 10 years old who suffered from various infectious diseases. They were even found in a 3½ year old child with measles followed by scarlet fever.

In 1909, Foster studied sections of aorta and arteries from persons varying in age from 6 months to 80 years and concluded that there is progressive development of elastic tissue up to 35 years of age, causing an increase in thickness of the wall of the blood vessel and a relative decrease in muscle tissue. Therefore, in the early decades, a moderate

increase in thickness of the walls of blood vessels due to physiologic processes is to be expected.

By this time, the term arteriosclerosis had come to include such a wide variety of lesions that different writers attempted to define and classify the various lesions. But opinions varied so widely concerning etiology, site of origin (in intima, media or adventitia) and the steps in development that no definition or classification met with unanimous approval (Klotz, 1906; Brooks; Poynton; Aschoff, 1908; Halbey; Nascher). These, however, were not the first attempts made to define the term. Every few years since Lobstein in 1834 used the name "arteriosclerosis" to designate a pathologic process, some one has attempted to bring order out of chaos by means of a definition or a classification, but only the broadest use of the term has stood the test of time.

Collins, in 1906, wrote a review of the various theories concerning arteriosclerosis, including experimental data, but he said little about the disease in children. The following year, Vollbrecht attempted to collect all the available material—"clinical, pathological and literary"—on juvenile arteriosclerosis for an inaugural dissertation. Including only cases in which the patients were of ages up to 36 years, he collected twenty-eight from the literature and twenty-one from the Leipziger Medical Clinic. All of those in which the patients were under 20 years of age, on which the original report verified the diagnosis of arteriosclerosis, are included in the present study.

In 1908, another review of cases from the literature was written by Fremont-Smith. He concluded: "In the previously detailed cases there appears a remarkable uniformity in pathological changes in a great variety of conditions. . . . The findings of Flexner in typhoid fever and of Wiesel in many infections are practically identical and point to a uniform primary medial degeneration in these diseases, and in certain other intoxications not of bacterial origin."

Many other writers of this period mentioned juvenile arteriosclerosis. Aschoff (1908) saw a typical lesion involving the mitral valve in a 1 year old child. Schlayer looked for it in 100 persons between 14 and 23 years of age and found it in 37. Frederich and Romberg saw it in young persons subjected to hard physical labor.

During these years there were also further studies made concerning the relationship between renal disease and arteriosclerosis. Miller and Miller and Parsons and Barber (1913) reported cases of chronic nephritis in children in which the vascular changes were slight or absent, while reports on other cases showed marked vascular involvement (Barber, 1913; Miller and Parsons). Gaskell classified nephritis

according to the type and extent of the vascular lesions. Ernberg made follow-up observations on a large number of cases of nephritis in childhood, ascertaining, among other things, that a considerable proportion of such cases reveal cardiovascular symptoms during the third decade of life. Most of his patients did not return for follow-up until after they were 20 years of age, but the extent of the disease in some of them would indicate that the cardiovascular involvement had begun years earlier.

In 1914, Stumpf studied the aortas of eighty-five children and found degenerative changes frequently after the first year of life. He wrote, "I have shown that the degenerative changes in the aorta of the child are in part due to mechanical factors. As I believe, it comes about in certain places by pressure and pull of developing disturbances in the circulation of lymph in the vessel wall, from which a degeneration of tissue, injured in this way, takes place. . . . As one studies many groups of children of different ages one must conclude that the mechanical factor is not the only one which may injure the aortic wall in youth." He believed chronic infections might also produce such changes.

During the same year, Holt said, "In early life chronic disease of the blood vessels is exceedingly rare. But even young children are not exempt." He found reports of seven cases of atheroma in the literature and believed that probably the most important etiologic factor was syphilis, but he mentioned several other possible contributory factors.

During the years from 1915 to 1930, interest in the problem of arteriosclerosis continued to grow, as is shown by the great mass of material in the literature on all phases of the subject. Reports of nineteen cases in persons under 20 years of age were published, and several studies are on record that lay special emphasis on the disease in children. Saltykow in 1915 sought to differentiate normal developmental changes in the aorta from degenerative ones, but was convinced that "the so-called fatty changes in the arteries of childhood and youth, especially in the aorta, are nothing else but the beginning of atherosclerosis." McMeans studied the changes in elastic tissue following acute infections in children as young as 5 years. He interpreted the changes as "a tissue response to an irritant," and described them as (1) granulation and splitting of the elastic fibers, (2) diffusion of the elastic-staining material and (3) difference in tingeability of the fibers. He believed that they pointed to a chemical rather than a mechanical change and were found in cases in which age or wear and tear could be factors. The elastic tissue gradually acquires a special affinity for fatty substances, which, in turn, predispose to calcification. Any cellular exudate present arises by a direct migration of wandering cells from the surface of the artery.

Klotz (1926) examined the arteries of young people (from 10 to 18 years of age) who had died of acute infections. He decided that the mechanical theory could not adequately explain the button-like thickenings in the intima.

In 1920, Evans reviewed arteriosclerosis as related to renal disease and found extensive evidence to support his theory that arteriosclerosis is of inflammatory origin. In the examination of 1,800 fixed sections, he found sclerosis of small blood vessels more common in the kidney than in any other organ, and never found it elsewhere unless present also in the kidneys and spleen. Two years later, he said that in younger persons the response to injury is apt to be more active, therefore the endothelial proliferation in the walls of small blood vessels is more marked in juvenile than in adult arteriosclerosis. "This," he said, "is further evidence of the inflammatory origin of arteriosclerosis." Evans considered the changes in the vessels and in the glomeruli as probably being simultaneous results of a single pathogenic agent. The same opinion has since been expressed by Dyke and others. Evans concluded that arteriosclerosis is not uncommon in children and is essentially identical with the process in adults, as is confirmed by (1) the nature of the lesion in the arterial wall, (2) its distribution in the vascular tree, (3) its incidence in the various organs and (4) its association with increased blood pressure and left ventricular hypertrophy.

During this period, experimental work followed many paths. Bailey produced extensive degeneration of the aorta and large vessels, as well as pronounced renal changes, by injecting large doses of diphtheria toxin. Schmidtman produced a rise in blood pressure accompanied by arterial changes in animals by feeding pulverized liver. Anitschkow and others fed animals high cholesterol diets and found fatty deposition in the deep layer of the intima. Newbergh and Clarkson found that a high protein diet would produce atherosclerosis in rabbits even when the cholesterol content was low. Alter used high protein diets and virulent bacterial capsules, obtaining intimal lesions in twenty weeks. Nuzum and his co-workers thought the acid-base equilibrium in the body was an important etiologic factor in their experiments. Ophüls (1921a), however, believed that none of these experimental lesions exactly duplicated human arteriosclerosis.

During the last ten years, several more reviews have been published, covering the subject of arteriosclerosis (Evans, 1923; MacCallum; Oertel; Aschoff, 1924; Clifford; Allbutt, 1925; Klotz, 1926). MacCallum remarked, in 1922, that "we are quite as ignorant of the underlying cause of arteriosclerosis as were our forefathers in the days of Morgagni." He also said, "Arteriosclerosis may be found in a highly

developed form in children." As factors in arteriosclerosis, Klotz mentioned diseases of childhood and adolescence, chronic intoxications, minor infections, overwork and fatigue, bacterial infections and certain exogenous poisons.

In 1927, Fishberg studied the arteriolar lesions in glomerulonephritis. He thought the probable sequence of events was: Chronic glomerulonephritis caused endarteritic obliteration in the vessels to the diseased glomerular tufts, followed by hypertension and generalized arteriolar lesions, which in turn caused widespread destruction of glomeruli, uremia and death.

Moschowitz and Cheney studied arteriosclerosis in the pulmonary circuit, but said little of its occurrence in childhood. The former mentioned congenital lesions of the heart as a possible factor in etiology.

In 1927, Moulonguet and Pavie differentiated "presenile arteriosclerosis" from syphilis, the former being a degenerative lesion, noninflammatory, with characteristic changes in the limiting elastica. According to Jeans and Cooke, syphilis is an uncommon factor in the causation of cardiovascular disease in children. Syphilitic lesions of the heart and large vessels are so rare, according to these authors, as to be pathologic curiosities, but lesions of smaller vessels are somewhat more common, especially in the central nervous system.

Joslin has written several articles in recent years on the association of diabetes and arteriosclerosis. In 1927, he reported arteriosclerosis in 20 per cent of patients between 10 and 19 years of age in whom diabetes developed. In 1929, he wrote, "The presence of arteriosclerosis was demonstrated by the roentgen ray in five of 29 diabetic children. The duration of the latter disease was five or more years."

During 1927, the association of arteriosclerosis with renal infantilism was discussed by Hunt. He reviewed fifty-four cases of the latter disease and reported arteriosclerosis in 10 per cent of cases that had come to autopsy. The sclerosis was limited to the larger vessels. He concluded, "In general, the vascular changes are much less pronounced than those found in adults with a similar degree of renal involvement."

In 1930, following the report of a case (table 1), Murphy remarked, "The development of a profound arteriosclerosis in these young patients who have diseases characterized by hypercholesteremia leads one to suspect that a high fat diet, one rich in cholesterol fat, may be more injurious than is commonly supposed."

Table 1 consists of ninety-eight cases of juvenile arteriosclerosis collected from the literature. An attempt was made to include all cases in persons under 20 years of age having the type of lesions described in the definition of arteriosclerosis given. In each case, the author's

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
1 5 yr. F	.....	.....	...	.....	Calcified temporal artery	.....	.....	Cited by Fremont-Smith *
2 9 yr. M	.....	.....	...	.....	Atheroma of aorta	.....	.....	Cited by Seitz (1896) *
3 8 yr. F	.....	.....	...	.....	Calcified plaques in aorta	.....	.....	Andrel (1829) *
4 10 yr. F	Shortness of breath for 5 yr.; no other illnesses	Extreme dyspnea; pallor, cyanosis	...	.....	Aneurysm of aorta	.....	.....	Roger
5 9 yr. F	.....	.....	...	Hypertrophy of left ventricle	Thickening of adventitia of arterioles of pia mater and kidneys	Small, granular 2 and 1½ oz. (56.4 and 42.4 Gm.)	Small, tough spleen	Gull and Sutton *
6 6 yr. F	Grandmother had chronic arthritis; patient had no childhood diseases, but always weak and delicate; treated for enuresis when 2 yr. old; convulsion 1 yr. before	Frequent headaches and earaches, albuminuria, recurrent convulsions	...	Hypertrophy of left ventricle	Renal arteriosclerosis	Small, granular	Pulmonary emphysema	Barlow *
7 2 yr. M	.....	Loud murmur over base of heart transmitted over thorax, exaggerated impulse, convulsions, smallpox	...	Marked hypertrophy, chronic pericarditis, aortic stenosis and insufficiency	Yellowish-white plaques, 2-5 mm. in diameter, in aorta	.....	.....	Moutard-Martin *
8 Still-born F	.....	.....	...	Dilatation of left ventricle	Aneurysm of aorta, 10 by 11 cm., arising below renal arteries and filled with blood clot; wall of sac contained coats of aorta with hyperplastic intima, fatty infiltration of muscle cells and mild inflammatory hyperplasia	.....	.....	Phänomenow *
9 12 yr. F	Scarlatina	Headache, coma	...	Enormous hypertrophy of left ventricle	Stiff cerebral arteries, marked thickening of muscular and fibrous coats	Chronic nephritis	Large hemorrhage in posterior lobe of left cerebral hemisphere	Dickinson *

10 14 yr. M	Renal stone at 3 yr.	"Wizened face and parchment complexion," headache, diminished vision, vomiting, polyuria, polydipsia, albuminuria, convulsions	...	Weight of heart 8 oz. (0.2 Kg.), left ventricle 1 in. (2.5 cm.) thick, mitral valve atheromatous	Atheroma of descending aorta and of coronary and cerebral vessels	Granular degeneration	Marked anemia of brain, ulcerative enteritis and peritonitis	Dickinson *
11 11 yr. M	Scarlatina 3 yr. before	Sick since scarlet fever 3 yr. before; headache, vomiting, drowsiness, edema, polyuria, diminished vision, pallor, wasting, epistaxis	...	Hypertrophy of left ventricle	Thickening of renal arteries	Granular with many petechial hemorrhages	Albuminuric retinitis with detached retina	Dickinson *
12 6 yr. F	Repeated colds	Dropsy for 2 yr.; albuminuria with blood and fat in urine	...	Hypertrophy of left ventricle, few atheromatous spots on mitral and aortic valves	Thickening of renal arteries	"Mottled tubal nephritis"	.....	Dickinson *
13 13 yr. F	.....	Uremia	...	Hypertrophy of left ventricle; weight of heart, 9 oz. (0.28 Kg.)	Thickening of pial arteries	"Tubal nephritis"	.....	Dickinson *
14 10 yr. F	.....	Renal dropsy for 3 mo.	...	Hypertrophy of left ventricle	Hypertrophy of muscle of cerebral arteries	"Tubal nephritis"	.....	Dickinson *
15 7 yr. M	.....	Sick 10 wk.	...	Marked hypertrophy of left ventricle	Marked fatty degeneration of pial arteries	"Tubal nephritis"	.....	Dickinson *
16 7 yr. M	.....	Severe scarlatinal dropsy, general edema, smoky urine, pneumonia	...	Hypertrophy of left ventricle	Hyaline thickening of pial arteries	Scarlatinal nephritis	.....	Dickinson *
17 12 yr. M	Weakly from birth	Palpitation, polyuria, apoplexy with left hemiplegia 1 yr. before, with headache, vomiting, loss of vision, oliguria, edema, nosebleeds, coma	...	Hypertrophy of left ventricle	Chronic endarteritis with medial hypertrophy of vessels of brain stem, aorta and brachials, arteriosclerosis (of Gull and Sutton)	Granular atrophy	Hemorrhage of brain, albuminuric retinitis	Filatoff *
18 5½ yr. M	Tuberculous peritonitis of long duration	Ascites, pallor, emaciation, diarrhea	...	.....	Fibrosis of splenic vessels	Interstitial nephritis	Atrophic cirrhosis of liver, spleen, kidney; tuberculous peritonitis (?) fibrous pleurisy; hypostatic pneumonia	Morell-Lavelee *
19 12 yr. M	Scarlet fever and dropsy at 8 yr.; rheumatism later	Dyspnea for 2 yr., headache, vomiting, drowsiness, fits, coma	...	Mitral insufficiency	Aneurysm of midcerebral artery; wall of vessel atheromatous and brittle		Hemorrhage into subarachnoid space and ventricles, adherent pericarditis, vegetative mitral endocarditis	Keating (1887) *

* Author indicates that a postmortem examination was obtained in this case.

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed—Continued*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
20 13 yr. F	.....	Sick 6 mo.; dyspnea, epistaxis, cyanosis	...	Marked hypertrophy of left ventricle, atheromatous deposits on aortic valve with aortic stenosis and insufficiency	Atheromatous aorta with dilatation of arch and small pouched aneurysm, the orifice of which was calcified	.....	.....	Sanne *
21 13 yr. F	Father syphilitic; patient had scarlet fever and psoriasis	Shortness of breath for 2 yr., pain in back, albuminuria, cyanosis, enlarged liver	...	"Colossal" heart, hypertrophy of left ventricle, grayish-white flecks in endocardium, normal valves	Narrowing of aorta for almost entire extent, inflammatory changes and thrombosis in abdominal portion, also narrowing of hypogastric, innominate, carotid and pulmonary arteries	.....	Amyloid in spleen and lungs (no evidence of syphilis)	Wallis *
22 8 yr. M	Improper feeding and excessive alcohol; family alcoholics	Sick 1 day (?); headache, dizziness, colic, convulsions, coma, cyanosis, diarrhea	...	Hypertrophy of left ventricle (2 cm.), "mother of pearl" spots on endocardium, also ecchymoses, early myocardial fibrosis	Atheromatous plaques and streaks in ascending aorta, renal arteriosclerosis, and arteriolosclerosis, coronary periarteritis and sclerosis	Albumin in urine 4 plus (post mortem)	Subpleural hemorrhage, acute emphysema	Girode *
23 15 yr. M	Mother neuropathic; patient had enlarged glands in neck and conjunctivitis; assisted alcohol vendor for 3 yr., and drank per day ⅗ glasses absinthe, 3 glasses whiskey, ⅗ liters wine and several glasses liqueur	Chronic alcoholism, loss of appetite, thirst, violent gastric pain, tremor, nightmares, repeated loss of consciousness, loss of breath on exertion, frequent epistaxis	...	Enlargement, accentuation of A2 and roughness at base	Hard, cylindric cordlike radials, distinctly felt ulnars, large, sinuous, hard temporals	.....	Extremities cold and blue	Girode *
24 5 mo. F	Deserted when 2-3 wk. old	Emaciation, marasmus; "looked like old dried up woman"	...	.....	Irregular thickening of coronary artery, involving the lining of the vessel with some extension to media	.....	.....	Meigs *
25 ? M	.....	Partial left hemiplegia, mental involvement, uniocular diplopia 2 yr. before, abscess in neck	...	.....	Thickening of cerebral arteries, with small aneurysm which had ruptured	.....	Large clot, right hemisphere with organization	Councilman *

26 4 yr. M	.....	Polyuria, headaches, decrease in weight, nausea, vomiting, diarrhea, thirst, diminished vision, uremia	...	.....	Thickening of renal arteries, especially of adventitia	Granular atrophy	Pulmonary congestion	Greene *
27 11 yr. F	Measles at 6 yr.; 2 attacks of influenza; no history of syphilis	Acute nephritis 3 wk., uremia (?)	...	Slight hypertrophy and dilatation of left ventricle, plaques in endocardium (like those in artery)	Involvement of all arteries of body; raised gray, translucent spots and patches; narrowed lumen especially in aorta, which hardly admits bullet probe at bifurcation	No scarring	No gummas, chronic perisplenitis, chronic perihepatitis, pneumonia, renal and pulmonary infarcts, hemorrhage into cerebellum	Hawkins *
28 14 yr. M	Chilblains of feet in winter	"Shingles," chest and back, prickly sensations left arm	...	.....	Absence of radial pulse on left, cordlike radials and brachials	.....	Slight cyanosis of fingers	Bond
29 12 yr. M	.....	Uremia	...	.....	Sclerosis of aortic arch, innominate artery and carotids	Contracted	.....	Simnitzky *
30 13 yr. F	Mother died at 36 yr. of phthisis; father living, aged 38; has catarrh and arteriosclerosis; patient's history showed measles, whooping cough and diphtheria, without nephritis	Following diphtheria, less than 1 yr. before, palpitation and dyspnea developed; followed for 5 mo. during which no change except occasional albuminuria	...	Enlargment, loud ringing, A2	Rigid radials and crurals, with decreased elasticity	Occasional albuminuria	.....	Seitz (1896)
31 14 yr. M	Father living, aged 44; has heart trouble; mother and sibs healthy; patient, measles, rheumatism in arms and legs	Palpitation and dyspnea on exertion, systolic murmur at apex	...	Enlargement to right, systolic murmur at apex, accentuation of A2	Rigid radials, also crurals and tibials, with decreased elasticity	No albumin	.....	Seitz (1896)
32 12 yr. M	Father died at 45 yr., had heart trouble; mother died at 39 yr., with marked arteriosclerosis; five healthy sibs; patient, severe scarlet fever and pneumonia at 5 yr.	Dyspnea on exertion since scarlet fever, also headaches and dizziness	...	Slight enlargement to left, ringing, clear A2	Very hard radials, visible pulsation in carotids and temporals, decreased elasticity	Slight albuminuria	.....	Seitz (1896)
33 9 yr. M	Repeated rheumatism	Heart trouble	...	Slight enlargement, hypertrophy of left ventricle, slight thickening of mitral valve, thickening of aortic valve with vegetations	Two patches of atheroma with outward bulging in aortic arch, small aneurysm in abdominal aorta at bifurcation, probably embolic in origin	.....	.....	Aitken *

* Author indicates that a postmortem examination was obtained in this case.

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed—Continued*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
34 13 yr. M	.....	Abdominal typhus	...	Heart pale and flabby, congenital anomaly in right atrium (reticulum), no hypertrophy, atheroma of aortic valve	Arteriosclerosis in ascending aorta, slight changes in all parts of aorta, yellowish-white streaks and plaques with thickened intima	.....	Typhoid fever, suppurating bronchitis, lobular pneumonia	Chiari *
35 11 yr. M	Measles and whooping cough	Bright's disease, vomiting, cough, pain in side, loss of weight, diarrhea with blood, edema of legs, albuminuria	...	Apex in 6th interspace, hypertrophy of left ventricle	Early atheroma at root of aorta	Weight 3 oz. (84.9 Gm.) together; granular, firm	Ascites, bronchitis	Russel *
36 14 yr. F	3 sibs have chronic nephritis; patient weak, undersized; had gastro-enteritis at 6 mo.	Shortness of breath on exertion, paralysis of left side following fright, with fever and hemoptysis	...	Marked enlargement of left ventricle, systolic murmur at apex and in the aortic area, accentuation and reduplication of A2, fibrinous pericarditis, moderate atheroma of aortic valve, slight thickening of mitral valve	Generalized chronic arteritis, patches of atheroma in aorta, especially around coronary orifices, marked atheroma of coronary artery with calcification	Chronic nephritis	Staph. albus septicemia; pulmonary edema; hemorrhage in lungs, spleen and mesentery; pneumonia; congestion of liver with calcification; acute splenitis; gastro-enteritis	Brill *
37 2 wk. ?	7 mo. gestation; poorly nourished	Generalized edema	...	No abnormality	Atheromatous plaques in pulmonary artery, as in a senile aorta, and brittle, calcified walls; rigid aorta with no gross lesions; microscopically, typical atheromatous plaques with calcification, more marked in pulmonary artery, with calcification	.....	Fibrinous peritonitis	Durante *
38 7 yr. F	Weak infant; diarrhea, measles	Frontal headaches for 2 mo., vomiting, development of hemiparesis, convulsions, uremia	...	Enlargement of heart, hypertrophy of left ventricle	Palpable brachials, arteriosclerosis, renal arteriolosclerosis	Albuminuria, diffuse interstitial nephritis	Cerebral hemorrhage	Greene *
39 10 yr. M	No evidence of syphilis; mother and father and 5 sibs living and well; patient—measles at 7 yr., chorea for 2 yr., nocturnal enuresis, thirst	Headache 1 wk., convulsions and vomiting, glandular swelling in right side of neck, bleeding into mouth, stomatitis ulcerosa, albuminuria, repeated epistaxis, development of systolic murmur at apex with signs of decompensation, constipation	...	Weight 8½ oz. (0.21 Kg.), granular patches in endocardium and at bases of great vessels, hypertrophy of left ventricle	Sclerosis of all vessels in the body, except cerebrals; all coats involved; general and coronary circuits (pulmonary ?) included	Chronic nephritis, with acute nephritis	Edema of ankles	Baines *

40 6 mo. M	Breast-fed only 2 mo.; no childhood diseases; no syphilis in history, but first child was stillborn	Loss of weight, weakness, constipation, development of diarrhea and vomiting; right foot became cold, then gangrenous	...	Slight hypertrophy of right ventricle, normal left ventricle, endocardium of left auricle hard, nodular, gritty; also that of left ventricle	Thick, cordlike femoral artery; normal cerebral artery; thick, rigid, nodular thyroid artery; tortuous, hard, thick coronaries; few atheromatous patches in pulmonary artery; sclerosis and grittiness of 1st part of aorta and bifurcation; rest not remarkable; sclerotic, obliterated iliac artery; sclerosis of femorals and branches, brachials, mesenteric, hepatics, thyroidal, renal splenic, etc.	Extreme phimosis, with marked dilatation of urinary tract and renal atrophy	No sign of congenital syphilis, gangrene of toes, caseous bronchial glands	Bryant and White *
41 7 yr. M	Weakly since birth	Patient underdeveloped, anemic, with diminished vision; polyuria, nocturia, headaches, edema	...	Hypertrophy	Marked thickening of blood vessels in kidneys	Advanced interstitial nephritis	Albuminuric retinitis	Greene *
42 17 yr. M	No evidence of syphilis; has had measles, tuberculosis	Patient not well enough to go to school for past 2 yr.	120	Slight hypertrophy	Thickening of radials and crurals	Normal	Apical tuberculous thickening (x-ray) with calcification of hilic lymph node, slight edema of extremities, congested liver	Hofbauer
43 8 yr. M	.....	Scarlet fever	...	Slight enlargement, pale muscles, plaques on mitral valve	"Chronic deforming endarteritis of the aorta," whitish-yellow plaques in intima of aorta; microscopically, thick intima with fatty cells in deep intima, also intercellular fat droplets, increase in connective tissue	.....	Obsolete apical and bronchial lymph node tuberculosis	Simnitzky *
44 12 yr. M	Inflammation of lung; trauma of leg	Suppurative osteomyelitis of femur, hemorrhagic infarcts in the lungs, seropurulent pleuritis, pyemia	...	Normal	Hard, raised plaques in aorta	.....	.....	Simnitzky *
45 17 yr. F	No history of previous illness	Cerebrospinal meningitis, fibrinopurulent rhinitis, suppurative bronchopneumonia	...	Normal size, anemic	Disseminated yellowish-white small patches in aorta	Chronic Bright's disease	Hydrocephalus, pulmonary edema	Simnitzky *
46 17 yr. M	No history of previous illness	Incarcerated herniotomy, lobular pneumonia, scoliosis	...	Normal	Hard yellow streaks in aortic intima	.....	.....	Simnitzky *

* Author indicates that a postmortem examination was obtained in this case.

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed—Continued*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
47 5 yr. M	Healthy	Pneumonia, vegetative endocarditis	...	Globular vegetations in right auricle (thrombosis)	Few hard white streaks in sinus of Valsalva and aorta	.....	.....	Simnitzky *
48 4 yr. M	Scarlet fever one month ago	Scarlatinal nephritis	...	Hypertrophy, thickening of mitral valve	Sparse grayish-yellow patches and streaks	Nephritis following scarlet fever	.....	Simnitzky *
49 10 yr. M	Healthy	Typhoid fever	...	Normal	Fine streaks in aorta	.....	Enlarged spleen, peptic ulcer	Simnitzky *
50 12 yr. M	Scarlet fever 2 yr. ago	Mitral and aortic insufficiency, chronic endocarditis	...	Hypertrophy of left ventricle, chronic endocarditis of mitral and aortic valves	Yellowish-white plaques in ascending aorta, arch and abdominal portion	.....	Congestion and edema	Simnitzky *
51 19 yr. M	Tuberculosis for 15 yr.; typhoid fever	Pulmonary tuberculosis with tuberculous enteritis	...	Streaking of aortic valve	Numerous longitudinal elevations of intima of aorta	.....	Tuberculosis	Simnitzky *
52 16 yr. M	Epilepsy	Epilepsy, pneumonia	...	Patches on aortic valve	Sparse elevated patches and streaks in intima of aorta	.....	.....	Simnitzky *
53 2½ yr. F	Pertussis 1 mo. before	Suppurative bronchopneumonia		Enlarged, pale flecks on aortic valve	White, thickened flecks in intima of aorta	.....	Edema of brain	Simnitzky *
54 14 yr. F	Epilepsy	Epilepsy, arrested tuberculosis of left node	...	Normal	Hard patches of thickening in intima of ascending aorta	.....	.....	Simnitzky *
55 18 yr. M	Articular rheumatism 6 yr. before; alcoholism	Heart disease	...	Chronic endocarditis of mitral and aortic valves with insufficiency and stenosis, thick pericardium, dilatation, hypertrophy	Numerous small grayish-white patches and streaks in intima of aorta and large vessels	.....	Congestion and edema	Simnitzky *
56 9 yr. F	Scarlet fever; tuberculosis	Tuberculosis	...	Normal	Hard raised patches in aorta	.....	Lymph node tuberculosis	Simnitzky *
57 18 yr. ?	Healthy	Abdominal typhoid, lobular pneumonia	...	Normal	Sparse patches and streaks in ascending aorta and arch	.....	.....	Simnitzky *

58 2 yr. M	Laryngeal diphtheria	Lobular pneumonia	...	Yellow plaques on aortic valve	Several small yellow plaques in aorta	.....	.....	Simnitzky *
59 17 yr. F	Tuberculosis	Tuberculosis	...	Normal	Raised yellowish-white flecks in entire aorta and branches	.....	Tuberculous laryngitis, tracheitis and lymph node tuberculosis	Simnitzky *
60 10 yr. F	Articular rheumatism 1 yr. before	.....	...	Chronic endocarditis of mitral valve, cardiac hypertrophy	Numerous small patches and streaks in aorta and large branches	Chronic Bright's disease	Arrested pulmonary tuberculosis	Simnitzky *
61 10 yr. M	Diphtheria 3 yr. before followed by otitis media	Sinus thrombosis following otitis media with operation, suppurative meningitis	...	Normal	Small, disseminated yellowish-white streaks and elevations in aorta and large branches	.....	Pulmonary abscesses, seropurulent pleuritis, pyemia, tuberculosis of bronchial lymph nodes	Simnitzky *
62 14 yr. M	Measles 6 yr. before	Suppurative periostitis and arthritis	...	Slight enlargement	Elevations around orifices and bifurcation of aorta and large branches	.....	Pulmonary abscesses and pyemia	Simnitzky *
63 13 yr. F	Scrofula	Chronic tuberculosis of lymph nodes, amyloidosis, anasarca, vegetative endocarditis	...	Hypertrophy of left side, globular vegetations in both ventricles	Raised patches and streaks in intima (aorta?)	Chronic Bright's disease	Amyloidosis, anemic infarcts in spleens	Simnitzky *
64 13 yr. F	Scarlet fever 8 yr. before	Abdominal typhoid fever, lobular pneumonia	...	.....	Small grayish-white patches in intima of aorta and large branches	.....	Decubitus	Simnitzky *
65 17 yr. M	Measles 8 yr. before	Postherniotomy peritonitis	...	Slight enlargement, pale muscle, few plaques on valves	Few plaques and streaks in intima of arch of aorta	.....	.....	Simnitzky
66 15 yr. M	Measles at 6 yr.; chickenpox at 8 yr.; polyuria for 1 yr.; recent headaches	Hemorrhage from gums following extraction, weakness, dyspnea, slight edema of ankles	...	Left ventricle 20 mm., right ventricle 9 mm., soft systolic murmur at apex; accentuation of A2, weight of heart, 300 Gm.	Decidedly atheromatous aorta with several calcified plaques in descending portion, spots of fatty degeneration in entire extent, fibrosis of renal vessels	Left, 68 Gm.; right, 80 Gm.; advanced stage of contracted kidney	Bilateral hydrothorax, congestion of lungs	Hirsch *
67 18 yr. F	Cancer of breast on mother's side; measles, pertussis, diphtheria; always weak; menstruated at 10 yr.; fall at 14 yr. and in bed 1 yr.; kidney trouble for 2 yr.	Development of about 14 yr., anemia, signs of nephritis, development of anasarca; patient died in coma	...	Left ventricle 15 mm., right ventricle 7 mm., accentuation of A2	Abnormally small aorta, no atheroma in aorta, marked thickening of renal artery	Chronic nephritis	Pulmonary congestion, old tuberculous lesion of spine with calcification	Hirsch *
68 11 yr. M	Scarlet fever; no other illness; no history of syphilis	Advanced arteriosclerosis	...	.....	Far-advanced widely distributed generalized arteriosclerosis	Chronic nephritis	.....	Anderson *

* Author indicates that a postmortem examination was obtained in this case.

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed—Continued*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
69 9 yr. F	Healthy	Headache, exhaustion, pain in cardiac region, dyspnea; patient died suddenly	...	Pericardium filled with blood clot	Infiltration of aortic sheaths with blood, dissecting aneurysm in intima with media, sclerosis only in two thickened areas, not at site of aneurysm, with microscopic evidence of proliferation of intima	.....	.....	Oppenheimer *
70 10 yr. M	.....	Patient died following resection of tuberculous joint	...	.....	Arteriosclerotic plaques in first portion of aorta	.....	.....	Oppenheimer *
71 4 yr. M	Father had gonorrhea before marriage, and syphilis (?); has been epileptic since age of 21; patient's birth weight 12 lb. (5.4 Kg.); required artificial respiration at birth; severe bronchitis at 6 wk. followed by whooping cough; fainting spell at 2 yr.	Systolic murmur since whooping cough, febrile attack like malaria but no improvement with quinine, increasing anemia, loss of weight, edema, sudden death	...	Blood-filled pericardiac sac, small, pale heart, aortic stenosis	Diminished radial pulse; aneurysm of arch and ascending aorta, ruptured into pericardial sac; extensive layers and patches of atheroma in aorta; very thick wall	Fatty deposits	Generalized glandular enlargement; large, hard liver and spleen; submucosal hemorrhage in stomach	Willson and Marcy *
72 11 yr. F	Measles at 2 yr.; headache and weakness 2 yr. before, with polydipsia, cystitis (?) enuresis	Enuresis, cystitis (?), dyspnea, headache, severe nosebleed, death	160	Aortic insufficiency, hypertrophy of left ventricle	Rigid radials and carotids, yellow marks in aorta and carotids, generalized (?) arteriosclerosis	Right, 120 Gm., with parenchymatous nephritis; left, 15 Gm.,; contracted	Hemorrhage in left cerebral hemisphere and into ventricles of brain, follicular cystitis, adherent pleuritis	Rach *
73 13 yr. M	.....	Patient somewhat small for age; had shortness of breath	120 75	.....	Rigidity of radials and other vessels (a constricted, diffuse rigidity)	.....	.....	Bahrdt
74 12 yr. M	Father, obliterating endarteritis, with gangrene, also syphilis	Pinched, haggard, looked like "old man"	...	Normal	Diffuse sclerosis of all palpable arteries with calcification and beading	Normal	Mentally bright	Fremont-Smith
75 6½ yr. F	Small baby; retarded growth and development and polyuria since birth	Development of child of 3 yr., edema of face and legs	...	Great enlargement, petechial hemorrhages, left ventricle ¼ to ⅜ in. (0.6 to 0.96 cm.), fibrosis of myocardium	Markedly thickened renal vessels, lumina narrowed, granular	Less than 1 oz. (28.3 Gm.) together	Bronchopneumonia	Miller and Parsons *

76 15 yr. F	Rheumatism on mother's side; several attacks of loss of speech and paralysis of legs lasting about 15 min.; bronchitis 2 yr. before	Cough, orthopnea on exertion and on exposure to cold; child small for age	...	Enlargement	Subclavian pulsations, sclerosis of brachials and radials, no pulsation in these	Trace of albumin	Congested fingertips, negative Wassermann reaction	Chapman
77 16 yr. M	Rheumatism; no history of syphilis	Patient dropped dead on street	...	Myocarditis, vegetation on aortic valve obstructing coronary orifice when valves were closed, obliterative pericarditis	Near occlusion of coronary arteries by sclerosis	.....	.....	*
78 15½ yr. M	Mother sick and overworked during pregnancy; no syphilis; polyuria for years	Stunted growth	...	Slight hypertrophy of left ventricle	Obvious arteriolosclerosis in the kidneys, no abnormality of aorta and arteries	Interstitial nephritis	.....	Barber *
79 13 yr. F	Lobar pneumonia several times in infancy	Postoperative intussusception, gangrenous appendix; patient died 6th day after operation	...	.....	Renal arteriolosclerosis with chronic periarteritis only in right kidney	Right, 9.6 Gm.; sligh acute nephritis, increased interstitial tissue, degeneration; left, 56 Gm.	Early generalized peritonitis	Simpson *
80 10 yr. ?	Chronic nephritis for 5 yr.	Chronic nephritis	...	Slight enlargement, grayish-white flecks on right side of heart	Yellow atheromatous changes in ascending arch and thoracic aorta with thick intima, also in carotids and innominate arteries, absent in other vessels	Chronic parenchymatous nephritis (no vascular changes)	.....	Hattori *
81 10 yr. F	Aunt an idiot; mother died in childbirth, had chronic nephritis; sister dead at 2½ yr. of renal disease; chronic nephritis diagnosed in patient at 1¼ yr.; retarded development	Idiocy, polyuria, albuminuria; patient died of diphtheria, with evidence of impending uremia	100	Enlargement, hypertrophy of left ventricle, two white plaques on mitral valve	Palpable arteries, renal arteriosclerosis and arteriolosclerosis	Chronic nephritis	Albuminuric retinitis	Glaser *
82 1¼ yr. M	.....	Renal dwarf with multiple fractures	...	.....	Great thickening of arteries	Fibrosis	General osteoporosis	Patterson *
83 14 yr. F	Weak and backward from infancy; severe headaches for years; measles in infancy; scarlet fever 2 yr. before; healthy family	Development of a child of from 5 to 6 yr.	240 190	Moderate hypertrophy of left ventricle, accentuation of A2	Small and wiry radial arteries, sclerosis of retinal vessels	Approaching uremia	Slight genu valgum, albuminuric retinitis, Wassermann reaction negative	Fletcher

* Author indicates that a postmortem examination was obtained in this case.

TABLE 1.—*Summary of Data on Arteriosclerosis in Young Persons in Cases Reviewed—Continued*

Patient, Age, Sex	Family and Personal History	Final Status	Blood Pressure	Observations in Heart	Blood Vessels	Kidneys	Other Observations	Source of Data
84 12 yr. M	Measles and scarlet fever at 3 yr.; hematuria at 9 yr.; enteritis 4 mo. before; frequent colds	Uremia, pulmonary edema	140 — 110	Moderate hypertrophy and dilatation of left ventricle	Slight arteriosclerosis	Late subacute nephritis (large kidneys mottled with hemorrhage)	Chronic suppurative tonsilitis	Ophüls *
85 14 yr. F	Weak since birth; scarlet fever 2 yr. before	Frontal headaches, enuresis, uremia (?), pain and deafness in right ear, coma, death	250 — 170	Hypertrophy	Sclerosis of peripheral vessels, retinal sclerosis with hemorrhage, marked sclerosis of aorta and large vessels of brain stem, obliterative endarteritis of renal arteries	Advanced chronic nephritis	Negative Wassermann reaction, renal dwarf, hypertrophy of suprarenal glands	Evans (1922) *
86 14 yr. M	Epilepsy 7 yr. before, following operation; mother had 4 miscarriages and 4 living children; measles at 4 yr.; bronchopneumonia at 5 yr.; tuberculous adenitis at 8 yr.; bronchitis annually	Defective vision, cerebral hemorrhage	195 — 170 260 — 200	Hypertrophy	Sclerosis of peripheral vessels, dilated aorta, arteriosclerosis with calcified plaques, arteriolosclerosis in kidneys	Chronic nephritis with tubular degeneration	Rickets, negative Wassermann reaction, albuminuric retinitis, cerebral hemorrhage	Evans (1922) *
87 14 yr. F	Scarlet fever 10 mo. before admission	Defective vision, headache, vomiting (1 wk.), uremia, stomatitis and terminal pericarditis	165 — 123	Hypertrophy of left ventricle, thickening of aortic and mitral valves	Thickening of renal arteries, other vessels not sclerotic	Extreme chronic nephritis	Albuminuric retinitis, pericarditis, hypertrophied suprarenal glands	Evans (1922) *
88 11 yr. M	Well until 2 wk. before	Septic sore on finger, development of uremia	190 — 120	Hypertrophy of left ventricle	Intimal atheroma of aorta, marked fatty changes in renal vessels with sclerosis of larger ones	Granular, scarred kidneys with punctate hemorrhages	.....	Dyke *
89 4 yr. F	Mother had 4 miscarriages; difficult feeding 1st yr.; frequent bronchitis; no other infectious disease	Drowsiness and vomiting following an eruption called "measles," development of crossed eyes, convulsion of left side, coma	...	Hypertrophy of left ventricle, atheromatous patches on mitral valve, thickening of aortic valve	Definite atherosclerosis in sinuses of Valsalva and ascending aorta	Chronic nephritis in scarred, granular, shrunken kidneys	Rickets	Greene *
90 10 yr. F	Always delicate; scarlet fever at 2 yr.; headaches since 6 yr.; treated for fits at 8 yr.; large healthy family	Nephritis, dyspnea, coma, convulsions	240	Marked hypertrophy of left ventricle, primary sclerosis of aortic cusps	Small, wiry pulse; carotid pulsations; tortuous retinal arteries with few hemorrhages; arteriosclerosis of aorta and coronary arteries; marked atheroma of abdominal aorta; renal arteriosclerosis (arteriolar)	Arteriosclerotic scars	Emaciation, purpuric spots on abdomen, negative Wassermann reaction, obliterative pleuritis, acute bronchitis, multiple small hemorrhages	Henderson *

91 3½ yr. M	Father's and mother's Wassermann reactions positive, but after birth of patient; patient had had measles, parotitis, diphtheria, scarlet fever and a tonsillectomy	Epigastric pain after meals, marked constipation, polydipsia, polyphagia, polyuria, enuresis, clotted blood in stools, death from hemorrhage	250 — 195	.....	Plainly visible temporal arteries; pipe stem radials; narrow, corkscrew retinal arteries, with fresh and old hemorrhages and degeneration	1,200 cc. of urine in 24 hr., heavy trace of albumin, few red and white blood cells	Old skin, wrinkled, brown, dry; liver 2 fingerbreadths below costal margin; negative Wassermann reaction (blood and spinal fluid); albuminuric retinitis	Schwartz
92 11½ yr. F	Measles and pertussis at 3½ yr.	Polyuria, marked emaciation, acute intussusception and gangrene of ileum	240 — 180	Apex in 6th interspace, weight of heart 240 Gm., enormous left ventricle, probable arteriosclerosis of mitral valve, atheroma of aortic valve	Atheroma of aorta and iliac artery (nodular), uniform thickening of systemic vessels, renal and splenic arteriolosclerosis	Congenital hyperplasia, subacute nephritis	Edema of lung with petechial hemorrhages, chronic passive congestion of liver and spleen	Schwartz *
93 2 da. F	Normal delivery	.....	...	.....	Circumscribed thickening of intima in axillary artery (fatty degeneration)	.....	Cause of death unknown	Hesse *
94 17 yr. M	.....	.....	184 — 122	Weight of heart, 550 Gm.	Arteriolosclerosis of kidney, spleen, pancreas, liver, lung	Diffuse glomerular fibrosis	.....	Branch *
95 6 mo. ?	.....	.....	...	Large septal defects	Isolated sclerosis of pulmonary artery (transposition of large vessels), lipoid degeneration most marked in smaller branches	.....	Negative Wassermann reaction	Watjen *
96 7½ yr. F	Father's Wassermann reaction positive; fracture of femur at 2¼ yr.	Genu valgum lordosis, tetany, cardiac decompensation, albuminuria, death; patient a renal dwarf	140 — 80	Dilatation, adherent aortic cusps, atheroma of endocardium	Atheroma of aorta	Chronic nephritis (contracted) kidney	Rickets marked by beading, bosselation, caries, stunted growth, dry skin; large fibrous spleen; fatty liver	Karsner *
97 4 yr. F	.....	Convulsions alternating with semistupor, sexual precocity, death following exploratory laparotomy	160 — 100	.....	Vascular sclerosis in pia	.....	Small thymus and thyroid gland, tumor nodules in lung, malignant hypernephroma, focal encephalomalacia	Dieterle *
98 16 yr. M	Tonsillitis and tonsillectomy at 7 yr.; cervical adenitis at 13 yr.; mastoiditis at 14 yr.; pneumonia at 15 yr.; epidemic meningitis at 15 yr.	Patient followed for 4 yr.; chronic nephritis with lipoid changes, hypercholesterolemia, convulsive uremia	160 — 110 250 — 130	Atheroma plaques on mitral valve	Atherosclerosis of aorta with thickening of vasa vasorum, renal arteriosclerosis and arteriolosclerosis	Chronic glomerular nephritis, lipoid degeneration	Albuminuric retinitis, anemia	Murphy *

* Author indicates that a postmortem examination was obtained in this case.

description of the lesion, when given, rather than his diagnosis, was taken as the criterion for including the case. There were several reasons for this procedure: In the early literature, lesions of syphilis and atherosclerosis were not clearly differentiated, and many lesions were described as lipoid degeneration in the intima, even with calcification, and then were called "syphilis." A few cases are included in which a history of parental syphilis was found, but the patients' lesions, as described, were those of atherosclerosis rather than syphilitic aortitis. On the other hand, many cases called "atherosclerosis" were rejected because the descriptions did not conform to the definition employed. Many of these rejected cases have been listed in studies of juvenile arteriosclerosis by other authors, and have been so frequently quoted and misquoted that it was deemed advisable to include them in a supplementary bibliography at the end of this paper.

Although an attempt was made to review all the literature on juvenile arteriosclerosis, it was found to be a well-nigh impossible task, since only a few cases are listed in the medical indexes under arteriosclerosis or even under vascular disease. A careful search through many reports of cases of chronic nephritis revealed a high incidence of arteriosclerosis. A similar review of certain other conditions might be just as fruitful.

Therefore, since this review is of necessity incomplete, the time is not ripe for final conclusions. A few comments, however, may be in order:

TABLE 2.—*Arteriosclerosis with Relation to Age*

Age of patients	Under 1 yr.	1-4	5-9	10-14	15-19
Cases	6	10	19	45	17
Percentage	6	10	19.5	46	17.5

Age.—Arteriosclerosis may occur at any age. The patients here represented ranged from the newly born to those 20 years of age. It is interesting to note that nearly half the total number of cases occurred during the age period of from 10 to 14 years, while a decidedly smaller number occurred in the succeeding age period of from 15 to 19 years. However, it may be that fewer cases in persons of the latter ages gain entrance to the literature, since the disease in the prepubescent person is so much more striking than it is in a person approaching adult life.

Sex.—There were forty-one females and fifty-three males, a difference too small to be considered significant in this review.

Family History.—No one factor appeared with sufficient frequency to be of value. In many cases, family history was not mentioned.

Past History.—Only seven patients were stated to have been healthy to the time of the final illness. In one or the other of the remaining ninety-one was found almost every illness of childhood. No one condi-

tion stood out prominently enough to signify etiologic value. The infectious diseases were of most frequent occurrence, but such is also the case in children without arteriosclerosis.

Blood Pressure.—When recorded, it was high. In most of these cases, however, chronic nephritis was present. In cases without renal involvement, blood pressure was usually not mentioned.

Heart.—There was cardiac involvement in sixty-eight cases. In twelve, the heart was stated to be normal and in eighteen it was not mentioned. The most common departure form normal was hypertrophy of the left ventricle. Atheroma or fibrosis of the endocardium was described in thirty-two cases. Most of these were fairly definite atherosclerotic lesions, although a few may have been scars of previous bacterial endocarditis.

Incidence of Vascular Lesions.—The incidence of vascular lesions in the cases was as follows: hypertrophy, 55; endocardial involvement, 32; normal heart, 11; heart not mentioned, 18; involvement of aorta and large branches, 59; of peripheral vessels, 25, and of visceral arterioles, 44.

In a few of the cases, the peripheral vessels were the only ones mentioned; in those coming to autopsy, the visceral and peripheral vessels were often neglected.

COMMENT

Perhaps more important than any conclusions to be drawn from this review are the unanswered questions that it has raised, a few of which are listed:

1. What is the real incidence of arteriosclerotic lesions in children dying from any cause? This question can be answered only by a careful review of autopsy material, both gross and microscopic, from a large series of cases.

2. Under what conditions, if any, do chronic renal lesions occur in children without lesions in the vascular system? What is the real incidence of renal lesions in juvenile arteriosclerosis?

3. Congenital malformations causing partial obstruction in some portion of the urinary tract are not rare. In what proportion of these cases are vascular changes found? Or does the case of Bryant and White represent a coincidence?

4. Does congenital or acquired syphilis ever produce typical atherosclerotic lesions with calcification?

5. Are vascular lesions any more numerous in children with poor heritage, many childhood diseases and faulty environment than in those showing an absence of these factors?

6. Is there a real peak in the incidence of arteriosclerosis around the onset of puberty? If so, why?

7. Would careful studies of metabolism reveal retention phenomena that would help explain the apparent frequency of association between renal lesions and arteriosclerosis, also between diabetes and vascular lesions?

8. Does hypertension without renal changes ever occur in childhood?

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Laboratory Methods and Technical Notes

NEW METHOD OF DECALCIFICATION*

NEWTON EVANS, M.D., AND ARAM KRAJIAN, LOS ANGELES

The following simple method has been developed for the preparation of bony and other calcified tissues for section.

The decalcifying solution consists of equal parts of (1) 85 per cent aqueous solution of formic acid and (2) 20 per cent aqueous solution of sodium citrate. This is used following the usual fixation of appropriately sized blocks of tissue in a diluted solution of formaldehyde U. S. P. (1:10) or other standard fixative. The decalcification requires from a few hours to three or four days, depending on the character of the tissue and the size of the blocks.

After decalcification is complete, the tissue must be washed in running water for from sixteen to twenty-four hours. Thorough washing in water is essential, otherwise the subsequent embedding or freezing preparatory to sectioning cannot be accomplished. The tissue is then cut by the freezing or by the paraffin method and stained in the usual manner.

The use of this method for a number of months in comparison with decalcification by nitric acid, makes evident that it has marked features of superiority over the latter method. The most prominent point is that the cellular elements are practically unaffected and take the usual stains apparently as perfectly as tissues that are not subjected to any decalcifying process. It is our observation that after tissues have been subjected to nitric acid, the nuclear elements do not take stains well, and the longer the time during which they have been exposed to the acid the less satisfactory are the results.

In a recent review of methods of preparing bony tissue, Jaffé [1] stated that formic acid is not a satisfactory agent because of its tendency to produce swelling of the fibers. Our observations fail to detect this defect. It is possible that the combination of the citrate with the formic acid counteracts such a tendency.

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* From the Pathology Laboratory of the Los Angeles County General Hospital.

1. Jaffé, H. L.: Methods for the Histologic Study of Normal and Diseased Bone, Arch. Path. **8**:817, 1929.

Notes and News

Society for Experimental Biology and Medicine, New York.—The Society recently elected Peyton Rous president, D. J. Edwards vice president and S. J. Goldfarb secretary-treasurer.

Institute for Advanced Study.—The initial endowment of this institute, which is founded by Louis Bamberger and his sister, Mrs. Felix Field, Newark, N. J., is $5,000,000. The aim is to provide facilities for research and training of advanced students by "eminent men of learning." The trustees for the first year have been selected; Abraham Flexner is the director of the medical division, and the further organization is in progress.

Gift to National Institute of Health.—The National Institute of Health (formerly the Hygienic Laboratory, Washington, D. C.) has been given $100,000 by the Chemical Foundation, Inc., it is reported, to be used for fellowships and studies in basic chemistry.

Cancer Institute Organized.—Under the direction of John G. William Greeff, commissioner of the department of hospitals, the Brooklyn Cancer-Radium Research Institute is being organized for special cancer work. John E. Jennings is chairman of the organizing committee, which will be a holding corporation for the distribution of scholarship and research funds and of funds where needed for cancer prevention.

Stokes Memorial Tablet.—The foyer of the municipal building in Baltimore has been selected for a memorial bronze bas-relief in honor of William Royal Stokes, chief of the bureau of bacteriology of the city health department for more than thirty years, who died Feb. 10, 1930, from psittacosis contracted while working on infected parrots.

Schaudinn Medal.—On March 23, 1930, the twenty-fifth anniversary of Schaudinn's discovery of the cause of syphilis, the Schaudinn Medal was awarded to F. d'Herelle, microbiologist, Yale University; Max Hartmann, protozoologist, Berlin, and Eduard Reichenow, protozoologist, Hamburg.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

DEPRESSED BONE MARROW FUNCTION FROM THE ARSPHENAMINES. DAVID L. FARLEY, Am. J. M. Sc. **179**:214, 1930.

Reports of seven cases of depressed function of bone marrow following treatment with arsphenamine are detailed. The clinical pictures presented by these patients varied according to the degree of the depression of bone marrow and according to the particular element or elements of the marrow most affected. The cases reported belong to the group of symptomatic blood dyscrasias. It seems likely that the direct cause is disintegration in vivo of the arsphenamines, so that a benzene-like action takes place. This, however, is a matter of opinion and not of proved fact. The rarity of occurrence suggests a preceding weakness of the hemopoietic apparatus in the persons affected. Careful examinations of the blood of patients showing unusual reactions to arsphenamine should be made. Blood transfusions should be vigorously repeated in the treatment of depression of bone marrow, keeping constantly in mind that a physiologic paralysis rather than an actual aplasia may be present in the particular case, and that the patient may be tided over this phase to recovery.

AUTHOR'S SUMMARY.

INTESTINAL PERMEABILITY IN OBSTRUCTION OF THE COLON. SIEGFRIED F. HERRMANN AND GEORGE M. HIGGINS, Am. J. M. Sc. **179**:365, 1930.

The general permeability of the wall of the colon to particulate graphite is not increased in obstruction under the condition of the experiments here reported. In the presence of injury to the mucosa and obstruction, however, particulate graphite may enter directly into the circulation and may be distributed by the portal blood stream.

AUTHORS' SUMMARY.

THE EFFECT OF INSULIN ON PATHOLOGIC GLYCOGEN DEPOSITS IN DIABETES MELLITUS. SHIELDS WARREN, Am. J. M. Sc. **179**:482, 1930.

Pathologic deposits of glycogen tend to disappear in cases of diabetes in which the patients are treated with insulin. The normal storage of glycogen is increased in diabetic patients by treatment with insulin. Sepsis decreases the effect of insulin so far as maintaining a normal distribution of glycogen is concerned. The distribution of glycogen may be a valuable aid in the postmortem diagnosis of active diabetes. Glycogen in the renal epithelium may represent an attempt at salvage of the carbohydrate being lost in the urine. Variations in deposits of glycogen in the skin of diabetic subjects may be related to their susceptibility to cutaneous infection.

AUTHOR'S SUMMARY.

EARLY CHANGES IN DOGS FROM STERILE EXTRACT OF ANTERIOR LOBE OF HYPOPHYSIS. EDWARD B. BENEDICT, TRACY J. PUTNAM AND HAROLD M. TEEL, Am. J. M. Sc. **179**:489, 1930.

The experiments reported here confirm our earlier observations on experimental hyperpituitarism and show particularly the early changes resulting from daily injection of a sterile active anterior lobe extract. After only three months of injection definite changes are noted in the thyroid gland and in the genital tract. The changes in the thyroid consist in hypertrophy of the gland epithelium with invasion of the lumen and almost complete absence of colloid. As to the genital tract, both male and female were sexually inactive. In the female there

is an hypertrophy of the uterus, ovaries and vagina. In the male there is no hypertrophy, the testicles being small and showing only the earliest stages of spermatogenesis. Skeletal overgrowth and splanchnomegaly were noticeable in the case of the foxhounds after three months of injection. Sluggishness, inalertness and plantigrade stance were all noted after two months of injection. A comparison was made between hypophysectomized and normal animals. Cessation of growth after hypophysectomy was confirmed. A marked slowing in the rate of growth of hair was noted. Normal alertness was not interfered with by hypophysectomy.

AUTHORS' SUMMARY.

THE OUTCOME OF 625 PREGNANCIES AFTER IRRADIATION. DOUGLAS P. MURPHY, Am. J. Obst. & Gynec. **18**:179, 1929.

The analysis is based on answers to questionnaires from all parts of the country. Only therapeutic irradiation was considered. The most striking outcome is the high frequency of gross deformities in the child after pelvic irradiation. The central nervous system was affected most often. The frequency and uniformity of the defects observed (microcephally most frequent) leave no doubt that irradiation of the internal genitalia of pregnant women is likely to be followed by seriously defective offspring.

A. J. KOBAK.

THE EFFECT OF PARATHORMONE ON NORMAL AND VITAMIN B-DEFICIENT RATS. W. B. ROSE AND C. J. STUCKY, Am. J. Physiol. **91**:513, 1930.

Because of a number of similarities in behavior in dogs suffering from parathyroid tetany and from deficiency in vitamin B, the hormone was tested for possible content of the vitamin complex. Using rats in which to a diet deficient in vitamin B was added a parathormone-dextrose, preparation prepared in such a way as to preserve any vitamin B that may have been originally present, or with some animals, supplementing the deficiency by daily subcutaneous injection of parathormone, it was found that the hormone was without effect in overcoming the vitamin deficiency. Old rats succumbed after the third injection of 25 units of the hormone. Young rats, on the other hand, survived four successive injections of a proportional dosage. The injections resulted in a rise of blood calcium by approximately 40 per cent.

H. E. EGGERS.

THE EFFECTS OF ETHYLENE ON THE RATE OF GROWTH AND FERMENT ACTION IN ANIMALS. A. D. HIRSCHFELDER AND E. T. CEDER, Am. J. Physiol. **91**:624, 1930.

In view of the fact that ethylene induces the rapid maturation of green fruits, the work here reported was undertaken to determine if it exerted a similar effect on animal growth, or on animal ferment activity. No stimulating effect on growth was observed in rats on the saturation of their drinking water with ethylene, nor by the addition to their air of ethylene in various concentrations. Its effect on various ferments was to cause an increased activity of amylase, but no effect was noted on pepsin, trypsin or liver lipase.

H. E. EGGERS.

EFFECTS OF PARATHYROID INSUFFICIENCY. J. N. ESAU AND O. O. STOLAND, Am. J. Physiol. **92**:1, 25 and 35, 1930.

In three articles dealing with the subject of parathyroid insufficiency, the writers report, in dogs, the following observations: An increase of inorganic phosphorus in the blood, with an increase of the total acid-soluble phosphorus which accompanied in amount the severity of the symptoms. However, in cases with mild symptoms of parathyroid deficiency with delayed onset, the blood phosphorus usually fell below normal. Low inorganic phosphorus in the blood, and

particularly a low content of acid-soluble phosphorus, were highly favorable to recovery. Magnesium lactate and morphine sulphate were found to have beneficial effects despite a secondary progressive rise in inorganic and acid-soluble phosphorus, presumably through depressant action on the respiratory center and other nervous mechanisms. No relationship could be established between the rate of fall of blood calcium and the type and severity of symptoms, although there was evidence of a relation to the time of onset. The behavior of the calcium and acid-soluble phosphorus compounds in the blood of parathyroidectomized dogs is attributed to a disturbance of phosphocreatine metabolism chiefly within the muscle fibers, which affects the calcium-potassium ratio, with an effect on the permeability and irritability of the tissues whereby there is an accumulation of otherwise nontoxic metabolites with access of these to the cellular protoplasm, this producing the various clinical manifestations of parathyroid insufficiency. Administration of parathormone was found to reduce the inorganic and acid-soluble phosphorus until the calcium rose much above the normal. Then there was a rapid rise of inorganic phosphorus followed by a marked increase in acid-soluble phosphorus usually until death ensued. The injection of liver extract into dogs with parathyroid insufficiency was found to produce marked visceral symptoms, which were accompanied by increased severity of the tetany, or by the induction of a brief period of tetany in dogs during the depression state. Following the visceral reactions there was a recovery from the tetany; when these failed to develop, there was no improvement after treatment with extract. While in the unbenefited animals there was a rise of blood calcium following the injection of the liver extract, in animals suffering from tetany, and in normal animals, the injection was followed by a slight lowering of blood calcium. Synchronously, with the visceral reactions there was a marked fall in blood pressure, and during the period of low pressure there was distinct reduction of coagulation time. The administration of the liver extract did not markedly prolong the recovery period of thyroparathyroid ectomized animals. In general, liver extract was not found to be as effective an agent in counteracting the toxemia of parathyroid tetany as were calcium and other substances.

H. E. Eggers.

The Effect of Removal of the Liver on the Formation of Ammonia. J. L. Bollman and F. C. Mann, Am. J. Physiol. **92**:92, 1930.

Following the removal of the liver in dogs there was a complete cessation of the formation of urea, and administration of ammonia was followed by its appearance as ammonium salts in the urine and tissues. So that removal of the liver is usually found to be followed by a considerable increase of blood and tissue ammonia. Much of this appears to come from the gastro-intestinal tract, and to be independent of the urinary ammonia, which, following hepatectomy, depends on acid-base equilibrium to about the same extent as in normal animals. After hepatectomy there is a progressive loss of the preformed urea, until, with minimal amounts in the urine, there is an accompanying decrease of urinary ammonia, along with the appearance of large amounts of amino-acids, uric acid and creatinine. If these substances are administered, they are without effect on urinary ammonia, but if urea is given, the urinary ammonia is markedly increased—evidence of the origin of ammonia in the urine from urea.

H. E. Eggers.

The Placental Transmission of Insulin from Fetus to Mother. G. T. Pack and D. Barber, Am. J. Physiol. **92**:271, 1930.

Anesthetized pregnant goats were subjected to laparotomy, and insulin was carefully injected into the palpated feti, care being taken to avoid loss in the peritoneal cavity. While the procedure was invariably followed by abortion, death of the feti being presumably due to hypoglycemic shock, such death did

not occur during the period of the experiment, as was determined by palpation and abdominal auscultation. Determinations of blood sugar in the mother established the fact that there was transplacental transmission of the insulin.

H. E. Eggers.

The Experimental Production of Xerophthalmia in Mice. E. Pomerene and H. H. Beard, Am. J. Physiol. **92**:282, 1930.

In mice fed on adequate diets containing casein, extracted (A and B free) casein, and edestin as the protein factor, and commercial crisco, or this aerated for sixteen hours at 140 F., it was found that commercial edestin contained no vitamin A, and while crisco contained enough of this vitamin to prevent xerophthalmia in mice, it was inadequate to protect rats in similar circumstances. The latter evidently require more of the antixerophthalmic factor than do mice.

H. E. Eggers.

The Physiologic Action of Rattlesnake Venom. H. E. Essex and J. Markowitz, Am. J. Physiol. **92**:317-345; 695-705, 1930.

In this series of eight articles the authors report the results of the observation of the effects of crotalin on living animal tissues. Its administration was followed by a sharp and profound drop in blood pressure, which by a series of eliminations was found to be of peripheral character. The fall in pressure is strikingly similar to that following injection of antigen into a sensitized animal. Injected into skeletal muscle, it greatly weakens the fatigue curve; in uterine muscle it causes a maximal contraction which differs from that of anaphylaxis in that it has a long latent interval, and desensitization is slight, requiring repeated doses of venom. In this condition, histamine is still capable of evoking a maximal reaction. Perfused lung becomes tremendously edematous. The addition of venom to blood, provided plasma unmodified by oxalate is present, causes swelling of the red cells to spherical form with later hemolysis, both of these changes being absent after removal of the plasma or its modification by the addition of oxalate. From tests of the action of the venom on protozoa, it appeared to be a nonspecific protoplasmic poison. As an indication of immunity to the venom its effect on blood pressure served as a satisfactory criterion, as did also its effect on corpuscular swelling. Both of these criteria would indicate that in dogs the acquired immunity is of rather short duration. Certain similarities are pointed out between crotalin intoxication and anaphylactic shock—the sharp fall in blood pressure, with initial splanchnic constriction followed by congestion; frequently lost coagulibility of the blood; a wheal reaction following intradermal injection of the crotalin, identical with that observed after the injection of histamine or of suitable antigen; evidence of bronchial constriction after the injection of the venom; maximal contraction of perfused uterine muscle; precipitation in dog serum incubated with small quantities of crotalin; constant rise of urinary bladder pressure after its injection in the dog. In view of the fact that most of the outstanding effects of histamine may be obtained with crotalin, the writers suggest that the effect of tissue extracts or allied substances may depend for their depressor activity on a principle that is neither histamine nor cholin. Crotalin contains no histamine, and yet it causes reduction of blood pressure, reddening and whealing of the skin, and contraction of perfused virgin guinea-pig uterus—the usually accepted criteria of histamine.

H. E. Eggers.

Effect of Isolation of Tail of Pancreas on Carbohydrate Metabolism. G. de Takats, F. Hannett, D. Henderson and I. J. Seitz, Arch. Surg. **20**:866, 1930.

Sugar tolerance tests were made on dogs whose pancreatic glands were divided with an electrocautery and then wrapped in omentum. The tests repeated at

intervals of from two to four weeks for several months showed a definite fall in blood sugar and an increase in hypoglycemia. Intravenous doses of dextrose showed that a larger than normal amount of dextrose per kilogram of body weight had to be given intravenously in order to produce glycosuria. These observations would suggest the possibility that mild pancreatitis set up by the stimulus of the operation results in a hypertrophy of islet tissue.

N. ENZER.

THE METABOLISM OF AMINO-ACIDS IN HEALTH AND DISEASE. LESLIE WITTS, Quart. J. Med. **22**:477, 1929.

The amino-acid glycine was administered orally in amounts of from 25 to 50 Gm. in from 10 to 15 per cent aqueous solution. Amino-acid nitrogen, non-protein nitrogen, uric acid, urea and sugar of the whole blood were determined at intervals after the ingestion of glycine and compared with the fasting levels. In a normal person, after the ingestion of 50 Gm. of glycine, the amino-nitrogen of the blood increases from the fasting level of 7 mg. per cent to about 12, reaching its peak in from one to four hours and returning to the fasting level in from six to eight hours. In thirty-one miscellaneous conditions without involvement of the liver, the average fasting level of amino-nitrogen was 6.3 mg. per cent. Lower values were found in myxedema and in one nervous patient. The values were slightly higher in uremia and in a case of leukemia. In twelve cases of disease of the liver, the average fasting level was 6.8 mg. per cent, and in ten of these the amino-nitrogen was between 5.7 and 8 mg. per cent. A low value of 5 mg. per cent was found in a case of Hanot's cirrhosis, and a high value of 8.6 mg. per cent in a comatose patient with cirrhosis of the liver. Following the ingestion of glycine, the curves were essentially similar to those obtained in controls. In diseases of the liver, with the exception of acute yellow atrophy, both the fasting and postabsorptive values of amino-nitrogen were normal. However, in acute yellow atrophy there is an increase in the amino-nitrogen of the blood, and a higher curve is obtained after the ingestion of glycine. This is probably due to the necrosis and autolysis of the liver cells. Following the ingestion of 25 Gm. of glycine by normal subjects, the blood urea increases from 30 per cent upward as high as 113 per cent. The same type of variation occurred in normal persons as well as in those with diseases of the liver. The blood sugar was found to rise after the ingestion of glycine, but no distinction could be made in diseases of the liver and in diabetes different from other diseases. In passive congestion of the liver, the protein metabolism appeared to be normal. In hypertension the amino-nitrogen was high and urea formation was deficient. In two diabetic patients, urea formation was defective, but in other cases of diabetes, in exophthalmic goiter, myxedema and gout it was within normal limits. The author concludes that tests based on changes in the blood amino-nitrogen or urea after the ingestion of proteins or amino-acids have no value in the diagnosis of hepatic disease.

N. ENZER.

THE FATE OF THYROXIN IN THE TREATMENT OF NEPHROSIS. R. PLATT, Quart. J. Med. **23**:129, 1929.

Clinical observations are to the effect that patients with nephrosis have a very high tolerance to thyroxin. A low metabolic rate does not seem to account for this unusual tolerance. Experiments were made to determine whether or not the nephrotic kidney allows rapid excretion of thyroxin. Groups of tadpoles were taken, and to the vessel containing one group was added the urine of a case of nephrosis receiving from 7 to 10 mg. of thyroxin per day. To group B the same amount of normal urine was added, in which had been dissolved thyroxin in the calculated concentration which the urine of the patient would have contained had the drug been excreted. Two control groups receiving thyroxin only and one receiving normal urine only were used. The results seemed to show

that nothing approaching the amount of thyroxin administered is being excreted by the patient in an unchanged condition. The author suggests that the thyroxin is rapidly distributed in nephrosis, or its action is inhibited. The inhibition does not seem to depend on the increased level of blood cholesterol. N. ENZER.

SOME FORMS OF PERNICIOUS ANEMIA WITH KNOWN ETIOLOGY. ALFRED FONTANA AND KARL LAGEDER, Virchows Arch. f. path. Anat. **273**:553, 1929.

The material of this extensive paper (50 pages) is furnished by five cases of anemia. Syphilis, tuberculosis, pregnancy, enteritis, hepatitis are considered the cause of the pernicious anemia in these cases. The morbus Biermer is only one manifestation of the much wider concept, "pernicious anemia." Pernicious anemia is a complex of symptoms which may be produced by different causes.

ALFRED PLAUT.

CONGENITAL ANOMALIES OF LIPOID METABOLISM. A. ABRIKOSSOFF AND H. HERZENBERG, Virchows Arch. f. path. Anat. **274**:146, 1929.

The so-called Christian type is not a disease by itself but a skeletal form of Niemann-Pick's disease. All congenital anomalies of lipoid metabolism (xanthoses) form one group. The anomaly may be more marked in the cholesterol metabolism, in the neutral fats, the phosphatides or the kerasin. There may be ectodermal, visceral or skeletal forms. These anomalies obviously are mutations. They represent an inherited genotypically fixed disease of the mesenchyme. The factors are recessive and become manifest only after inbreeding. The hypercholesterinemia is not primary.

ALFRED PLAUT.

REMARKS ON ARTICLE BY ABRIKOSSOFF AND HERZENBERG. L. PICK, Virchows Arch. f. path. Anat. **274**:152, 1929.

The three types, Gaucher's disease, Niemann-Pick's disease, and the disease described by Christian, are different manifestations of a primary disturbance of lipoid metabolism. Nevertheless, each of the three is a definite clinical and anatomic entity. The clinical course of Niemann-Pick's disease generally is too fast for the development of a skeletal form. Intermediate forms must be expected. So far, about fifty cases of Gaucher's disease are on record and about fifteen of morbus Niemann-Pick and Christian's syndrome. In comparison with these figures, the number of intermediate forms is negligible.

ALFRED PLAUT.

THE INCREASED FREQUENCY OF THROMBOSIS AND EMBOLISM. H. AXHAUSEN, Virchows Arch. f. path. Anat. **274**:188, 1929.

Statistical survey of 11,266 autopsies between 1912 and 1928 showed a decrease in thrombosis and embolism up to 1921; then increase beginning in 1923. Since circulatory diseases in this study give figures not dissimilar to those of other disease, one cannot attribute the increased frequency to intravenous therapy. Surgical and medical cases show no difference. Obviously, we are facing a problem of changing frequency as it exists in many other diseases.

ALFRED PLAUT.

Pathologic Anatomy

THE LOCAL EFFECT OF THE INJECTION OF GASES INTO THE SUBCUTANEOUS TISSUES. A. W. WRIGHT, Am. J. Path. **6**:87, 1930.

By the subcutaneous injections of oxygen, nitrogen and carbon dioxide, there have been produced large numbers of monocytes, modified monocytes, epithelioid cells and epithelioid giant cells which resemble those of tuberculosis as seen in

supravital preparations. The causes for this reaction are nonspecific. Monocytes are considered to arise locally, originating from some type of fixed connective tissue cell. Epithelioid cells and epithelioid giant cells appear in these experiments to arise almost entirely from monocytes, the cause of the transformation being evidently due to some chemical change in the medium about the cell. Histologic structures resembling true tubercles have been found in considerable numbers. Fibrin is present in the gas spaces, often forming a thin membranous lining. The new formation of elastic tissue is suggested about gas spaces where tissue cells are under tension. Spaces lined with flat, mesothelial-like cells and containing both gas and fluid are found after six or eight days. The lining cells are thought to originate from connective tissue cells, although endothelium cannot be ruled out as a source.

AUTHOR'S SUMMARY.

MIXED TUMORS OF THE PALATE. R. D'AUNOY, Am. J. Path. **6**:137, 1930.

The term mixed tumor as applied to the neoplasms occurring in the general oral-facial region is distinctive and descriptive and should be retained. The origin of these tumors can be most satisfactorily explained by the theory of embryonal enclavement. Microscopically complex but clinically benign, it is doubtful if typical mixed tumors ever undergo so-called malignant changes. Certainly such transformations, if occurring, are difficult of proof. Palatal mixed tumors show the same general histologic complexity and clinical characteristics as those occurring in other regions. Two mixed tumors of the palate are reported.

AUTHOR'S SUMMARY.

PRIMARY MYOCARDIAL AMYLOIDOSIS. R. M. LARSEN, Am. J. Path. **6**:147, 1930

The distribution of amyloid within the myocardium in primary myocardial amyloidosis may be diffuse as well as focal. Its deposition within the heart may occur in the presence of hypertrophy as well as atrophy. It is deposited only in those tissues that have a known vascular bed. Its presence in avascular tissue is only accomplished by continuity with deposits in vascular tissue. The deposition of amyloid occurs primarily about venocapillary endothelium from which it extends to surround the normal tissues, ultimately cutting off the vascular supply to the part. Then only the tissues atrophy and are replaced by amyloid. This constitutes the primary mode of amyloid infiltration. Amyloid gains entrance to occasional cardiac muscle cells by a process of invagination and ultimate penetration of the cell wall. This is a direct method by which myocardial cells may be replaced by amyloid. There is no evidence that amyloid deposit is dependent on localized metabolic changes, nor is there evidence of primary pericellular deposition of amyloid, from which it freely invades living cell substance. The deposit of amyloid apparently is dependent on changes in endothelium, especially of venocapillaries, which may possibly become impermeable to some substance in the tissue lymph that may normally be present in tissue lymph and capable of permeating venocapillary endothelium.

AUTHOR'S SUMMARY.

GENERALIZED AMYLOIDOSIS OF THE MUSCULAR SYSTEMS. SHIELDS WARREN, Am. J. Path. **6**:161, 1930.

A case of generalized amyloidosis of the muscular systems is reported. The evidence indicates a widespread perversion of fibroblastic function. The parenchymatous organs are not involved.

AUTHOR'S SUMMARY.

AGE OF AMPHOPHILE LEUCOCYTES IN RABBITS. EMILY HUNT AND H. G. WEISKOTTEN, Am. J. Path. **6**:175 and 183, 1930.

The Arneth count made from smears from the blood of the rabbit is of definite value in determining the relative age of the amphophils, and that a "shift to the

left" in the count (increase in per cent of the simpler formed nuclei) actually indicates a relative increase in the number of young or more immature amphophils in the circulating blood. Cessation of the supply of amphophils from the marrow results in practically complete disappearance of amphophils from the circulating blood in a period of between three and four days. The average duration of the life of amphophils in the rabbit's blood is between three and four days.

AUTHORS' SUMMARY.

COMPLETE SITUS INVERSUS OF THE VENA CAVA SUPERIOR. B. HALPERT AND F. D. COMAN, Am. J. Path. **6**:191, 1930.

A rare vascular anomaly in a negro infant 2 weeks of age is described. The arrangement and the course of the dural sinuses, the large venous trunks of the neck, the vena cava superior and the vena azygos presented a mirror image of the normal. There was no trace of a right vena cava superior. Previous reports of this anomaly are reviewed.

AUTHORS' SUMMARY.

THE TOTAL NUMBER OF GLOMERULI IN THE CONGENITALLY ASYMMETRICAL KIDNEY. R. A. MOORE, Am. J. Path. **6**:199, 1930.

The enlarged kidney associated with hypoplasia or agenesia of the opposite kidney contains the usual number of glomeruli characteristic for one kidney of that species.

AUTHOR'S SUMMARY.

THE VASCULARIZATION OF THE EPICARDIAL AND PERIAORTIC FAT PADS. H. F. ROBERTSON, Am. J. Path. **6**:209, 1930.

The arteriae telae adiposae of the heart and ascending aorta proliferate in response to disease, augmenting the myocardial blood supply or tending to compensate any deficiency in it. The periadventitial vessels of the ascending aorta, joining the coronary vessels with those of the thorax, may greatly assist in this compensation. The fat bodies develop about the proliferating vessels and their size depends primarily on the extent of vascularization present, secondarily on such factors as atrophic and sclerotic myocardial changes, and individual peculiarities in local and general metabolism.

AUTHOR'S SUMMARY.

CHANGES IN THE FINGER NAILS IN PULMONARY TUBERCULOSIS. ALBERT G. HAHN, Am. Rev. Tuberc. **20**:876, 1929.

Pittings or depressions in the finger-nails were observed in 100 per cent of a group of patients suffering from active pulmonary tuberculosis as compared to 6 per cent of a group of patients in whom the tuberculosis had been inactive for a relatively short period, and a third group of patients without symptoms of activity for from one to twenty-five years in whom this change was absent in 100 per cent. These characteristic pittings in a known case of pulmonary tuberculosis are considered indicative of recently active tuberculous disease provided no other disease is present. Hippocratic incurvation was found in 76 per cent of the active tuberculous group, 50 per cent of the inactive tuberculous group, and 30 per cent of the ex-patient workers at the sanatorium. This change did not occur in any of the nontuberculous controls (presumably normal persons). Cyanosis of the finger-nails was noted in 66 per cent of the active group as against only 2 per cent of the inactive or chronic group. This change was well marked in all cases in which the disease was rapidly advancing, as evidenced by clinical symptoms and roentgenographic studies. Cyanosis may be of value in prognosis. Ridging seems to be of less importance than the other changes described.

H. J. CORPER.

COMPARATIVE RADIOGRAPHIC AND ANATOMICAL STUDIES OF INTESTINAL TUBERCULOSIS. M. MAXIM STEINBACH, Am. Rev. Tuberc. **21**:77, 1930.

On the basis of sixty-seven cases of far advanced pulmonary tuberculosis, studied clinically, roentgenologically and at autopsy in relation to tuberculous ulceration of the intestines, it was found that the roentgenologic signs usually considered diagnostic of this condition were highly unreliable in over 52 per cent of the cases. Microscopic as well as gross anatomic examination of the intestines should be done in all cases. Examination without incising the intestine is not to be relied on, since many of the early ulcerations involve only the mucosa and submucosa, and are to be detected only after opening and washing the intestines. A large number of ulcerations not visible macroscopically are seen only after careful microscopic search.

H. J. CORPER.

CHRONIC GLOMERULONEPHRITIS WITH LIPOID CHANGES. FRANCIS D. MURPHY, Arch. Int. Med. **45**:23, 1930.

The clinical features of a patient, aged 12 years, with chronic glomerulonephritis and lipoid changes in the various organs, are described from the onset of the disease until death five years later. During the course of the disease it is seen that the symptoms were evanescent and varied so much from time to time that the proper interpretation of the clinical picture was difficult in some stages of the disease. Lipoid deposits in the various parenchymatous organs are described, and their relationship to hypercholesterolemia is discussed. The atherosclerosis of the aorta and mitral valve is described and its dependence on hypercholesterolemia is pointed out. A description is given of the changes found in the arteries and arterioles of the kidney and other parenchymatous organs as well as those found in the skeletal muscles. The damage found in the renal arterioles is especially dwelt on.

AUTHOR'S SUMMARY.

HEMOCHROMATOSIS. ELMER H. FUNK AND HUSTON ST. CLAIR, Arch. Int. Med. **45**:37, 1930.

A case of hemorchromatosis without diabetes is reported. The copper content of the liver was found to be 140 mg. per kilogram of fresh tissue, or 331.8 mg. for the entire organ. The copper content of the spleen was found to be less than 1 mg. for the entire organ. The estimated quantity of iron in the liver is probably inaccurate but was found to be at least 6.2 Gm. per kilogram of tissue, or 14.7 Gm. for the entire organ. No analyses were made for zinc, manganese, nickel or other heavy metals. It is unlikely that increased hemolysis is the cause of the iron pigmentation. It is likely that the rôle played by copper is secondary to an unknown etiologic agent.

AUTHORS' SUMMARY.

ATELECTASIS IN THE PATHOGENESIS OF ABSCESS OF THE LUNG. THEODORE S. MOISE AND ARTHUR H. SMITH, Arch. Int. Med. **45**:92, 1930.

Obstructive atelectasis is a common observation in otherwise healthy albino rats. The obstruction is frequently the result of the accumulation of mucus in the form of a plug completely occluding the bronchial lumen. Micro-organisms may be frequently obtained from the lungs of healthy animals. On the basis of these observations, it is probable that the sequence of events in the pathogenesis of pulmonary disease in the white rat is the entrance of organisms into the lung, an obstructive atelectasis followed by the growth of organisms distal to the point of occlusion and the development of pulmonary suppuration. The established suppurative process may extend to other parts of the lung by a repetition of these changes or by direct extension to adjacent structures.

AUTHORS' SUMMARY.

THE RELATION OF THE DISTRIBUTION AND STRUCTURE OF THE CORONARY ARTERIES TO MYOCARDIAL INFARCTION. MERRITT B. WHITTEN, Arch. Int. Med. **45**:383, 1930.

The deep branches of the arteries of the left ventricle leave at right angles and pass directly through the myocardium. The branches of the arteries of the right ventricle spread out in practically the same plane as the larger artery from which they arise. Three distinct types of lesions are produced by infarction involving the left ventricle. The nature of the infarction depends on the site of the occlusion. The fact that the injury in infarction is almost always to the left ventricle, whereas the right ventricle rarely is involved, seems to depend on the differences in the anatomic structure of the arteries of the two ventricles. Infarction in the posterior surface of the left ventricle is much more common than has been heretofore recognized. Infarction at the apex may be due occasionally to occlusion of the right coronary artery. It is suggested that the position of the first part of the circumflex branch of the left coronary artery, while it is in the coronary sulcus and above the ventricle, is a factor in making it less liable to occlusion than the anterior descending artery. Infarction in the right ventricle was found only in connection with massive infarction of the left ventricle and usually was minimal in amount. The right ventricle, although it appears to be less vascular than the left, is not believed to be especially predisposed to failure with age. In fact, the left ventricle is found to be the one to fail most frequently from arterial insufficiency.

AUTHOR'S SUMMARY.

ACUTE, TOXIC (NONSUPPURATIVE) ENCEPHALITIS IN CHILDREN. A. A. LOW, Arch. Neurol. & Psychiat. **23**:696, 1930.

Low studied in detail the structural changes in the brains of children who died after a short course with manifestations of severe lesion of the brain, such as convulsions, coma, delirium, general spasticity, ocular nerve palsies and similar signs. The duration of the illness was two, four, ten, fourteen and thirty-nine days. Some structural changes in all of the five cases, in spite of the varied time element, were common. Here Low includes the absence of mesodermal elements (infiltrations) in the perivascular spaces and of hemorrhagic foci; extensive damage to the cortical ganglion cells (mostly as peracute or acute liquefaction); progressive glial and vascular reaction; the presence of so-called glial reticulum and a moderate meningeal reaction. In the five cases the changes varied somewhat. In the case, for instance, that lasted only two days, the ganglion cells showed so-called peracute liquefaction with disintegration, while the glia cells also showed regressive changes which in the corpus striatum were of the so-called ameboid type; in the cases that lasted two or more weeks, the glial changes were proliferative in the form of rich cytoplasmic glia, glia rosets, neuronophagia and satellitosis; the ganglion cell destruction was milder; the blood vessels were engorged and new capillaries were numerous. Low contrasted his observations with those of Lothmar and Rosenthal, who by injecting dysentery toxin and guanidin into rabbits obtained changes ilke those described by Low. The changes in the animals differed according to the virulence and the amounts of the toxins injected.

GEORGE B. HASSIN.

TUMOR OF THE BRAIN WITH SUDDEN ONSET OF SYMPTOMS. C. W. IRISH, Arch. Neurol. & Psychiat. **23**:727, 1930.

Tumors of the brain often give a clinical picture of an acute onset and rapid course much resembling a vascular or an inflammatory condition of the brain. Structurally, such tumors, in the greatest number of cases, were spongioblastomas, a type of glioma especially well studied by Globus and Strauss. They are malignant gliomas because of the rapid growth, as evidenced by the presence of numerous mitotic phenomena in the tumor cells and the presence of well defined

spongioblasts. Irish gives clinical histories of ten cases, nine of which proved to be definite spongioblastoma multiforme; one case was classified as medulloblastoma. The clinical picture varies, depending on the localization which in the series of Irish involved the frontal lobes (twice), frontotemporal (twice), temporal and occipital lobes (once in each), and once the vermis of the cerebellum. In three cases the lesions were multiple involving among other structures also the basal ganglions. The termination is practically always fatal, the entire duration of illness averaging fifty-eight days (from seven days to four months).

GEORGE B. HASSIN.

ELEPHANTIASIS AND EDEMA. F. L. REICHERT, Arch. Surg. **20**:543, 1930.

Emphasis is placed on the importance of appreciating that obstruction of the lymphatic and venous drainage does not cause elephantiasis unless there is a concomitant inflammatory process in the subcutaneous tissue. In a previous report by the author (*Arch. Surg.* **13**:871, 1926), there was reported the technic of complete severance of the tissue of the thigh, with the exception of the femoral artery and vein. Invariably edema developed in the extremity and an anastomosis could be demonstrated by opaque material injected into the arterial and venous system. Similarly, lymphatic regeneration could be demonstrated. Subsidence of the edema occurs with the regeneration of the lymphatic system, and edema persists if the lymphatics are blocked. The author attempted to superimpose an infection on such an edema, but all attempts by injecting various strains of streptococci failed, except for one instance. The author believes that so-called elephantoid conditions differ from true elephantiasis only in their extent. In contrast with simple edema, elephantiasis and elephantoid states present an entirely different picture in roentgenograms of the soft tissue. Not only do they show great thickening of the dermis and marked enlargement of the subcutaneous tissues down to the muscle, with a thickened muscular aponeurosis, but there is an extensive network of fibrous trabeculations in the hypodermal layer. The article is extensively illustrated by case histories and photographs of roentgenograms. No edema, even of long duration, will lead to proliferative change and deposition of fibrous connective tissue unless it is associated with inflammation. Lymphedema and lymphaticovenous stasis occurred in replanted limbs and in venous and perivenous blockade. The factor of lymphangitis in elephantiasis could not be reproduced experimentally. The elephantoid state is a sequel to varicose veins, phlegmasia alba dolens and to chronic bacterial fungoid and malignant ulcerations.

N. ENZER.

AMYLOIDOSIS OF THE THYROID GLAND. W. C. HUNTER and D. B. SEABROOK, Arch. Surg. **20**:762, 1930.

The authors report an instance of enlargement of the thyroid due to amyloidosis. The patient had advanced tuberculosis in both upper lobes. The thyroid was causing pressure on the trachea. Grossly, the gland was yellowish white, and somewhat soft and avascular; the colloid was scanty and vacuolated; there was a strongly positive test for amyloid, and there was a marked reduction in the number of acini; the epithelium was flat or cuboidal. There was a large amount of homogeneous hyaline substance between the acini. A review shows that the lesion is rather rare, although the author does not report the total number of cases on record to date.

N. ENZER.

ANNULAR PANCREAS. N. J. HOWARD, Surg. Gynec. Obst. **50**:533, 1930.

After dense adhesions between the gallbladder, omentum and the duodenum were separated it was found that the head of the pancreas completely encircled the midpart of the second portion of the duodenum. The pancreatic tissue was narrowed to an isthmus about 3 cm. broad at the lateral anterior wall of the

duodenum. Proximal to the constricting ring, the duodenum was dilated to a diameter of 6 cm. and distally constricted to 4 cm. The cutting of a good sized duct in the pancreatic tissue anterior to the duodenum led to the formation of a pseudocyst which on incision resulted in a pancreatic fistula. At least 1,100 cc. of fluid was discharged a day, but there was no intense ulceration of the skin which was attributed to the absence of duodenal secretions, which activates the pancreatic enzymes. The literature and the embryologic explanations of this anomaly are given.

RICHARD A. LIFVENDAHL.

PSEUDOTUBERCULOUS SALPINGITIS. J. DENTON and G. DALLDORF, Surg. Gynec. Obst. **50**:663, 1930.

It is believed that a foreign body type of reaction in the oviducts is frequently diagnosed as tuberculosis. In seventy-eight cases previously diagnosed as tuberculous salpingitis, there were thirty-four in which this confusing type of reaction had occurred. The foreign substance consisted of calcium refractive crystalloid clusters in the centers of basophilic masses and in the bodies of giant cells and endothelial nodules. These chemical substances are absent in true tuberculous lesions and the tubes are larger, cannot be easily separated from the surrounding granulation tissue and extensive caseation is present through the muscularis, and the tubercles on the serosal surface are confluent and caseous.

RICHARD A. LIFVENDAHL.

SOLITARY CYSTS OF THE KIDNEY. A. B. HEPLER, Surg. Gynec. Obst. **50**:668, 1930.

On a basis of four serous and three hemorrhagic solitary cysts studied by the author, a review of the literature and experimental work, the conclusions are reached that tubular block alone cannot produce such cysts, but if the same condition which produced tubular obstruction also interfered with the blood supply to the same segment of the kidney, thus resulting in parenchymal anemia and degeneration, then tissue relaxation, rapid dilatation and cyst formation might occur.

RICHARD A. LIFVENDAHL.

THE CAUSES OF NEONATAL DEATH. J. N. CRUICKSHANK, M. Res. Council, Special Rep. Series, no. 145, 1930.

In 800 autopsies in cases of neonatal death the cause of death was considered to be due to birth asphyxia, birth injury or prematurity in 540 instances, to infected conditions in 238, and to gross developmental defects in 22.

ACUTE EOSINOPHILIC LEUKEMIA AND EOSINOPHILIC ERYTHRO-LEUKEMIA. J. HAY and W. H. EVANS, Quart. J. Med. **22**:167, 1929.

The authors report two cases, the first of which was clinically identical with acute myelogenous leukemia, splenomegaly and general glandular enlargement and leukocytosis being present. The internal organs showed intense infiltration with eosinophilic cells. The white blood count in this case was 72,187, with 83.7 per cent eosinophils.

The second case was more chronic and showed the combination of polycythemia with leukocytosis and also a high eosinophilic count. Details are given of the clinical and the postmortem observations, and fairly complete records of similar cases selected from the literature. All of the cases are identical in that they closely resemble a myelogenous leukemia, with the exception that the cellular infiltrations are polymorphonuclear or adult eosinophilic cells.

The authors hold that there is no justification for the establishment of a new clinical entity, as favored by some authors, under the caption "hyperleucocytosis with splenomegaly." They urge, rather, that these are examples of myelogenous leukemia, differing only in the type of cell predominant. With reference to the

polycythemia and leukocytosis, they point out that in other cases of this type myelogenous forms appear in the circulation, and in one case (Blumenthal's) there was a frank combination of leukemia and polycythemia. Hence they favor the term "erythro-leukemia."

N. ENZER.

STEREOSCOPIC RADIOGRAPHY OF THE CORONARY SYSTEM. J. S. CAMPBELL, Quart. J. Med. **22**:247, 1929.

This is an instructive article on the blood supply of the heart. An opaque medium "Roentyum" was injected into the vessels, through each coronary artery. Stereoscopic x-ray pictures were then taken of the specimens. The results bear out the previous work of Gross. The author points out the inconstancy of the blood supply. His studies, based on 100 specimens, disclosed numerous variations in the distribution of the coronary vessels. In the auricles particularly he found nothing constant in the distribution of the vessels, with the exception of one branch of the ramus ostei cava superiosus. While this vessel has no constant origin, it has a constant termination. It helps to form the anastomosis at the junction of the superior vena cava with the right atrium. The ventricular blood supply is fairly constant on the anterior surface, but not so on the posterior surface. With regard to the blood supply of the neuromuscular tissue, it was found that the supply came in 81 per cent from the right coronary and in 19 per cent from the left coronary. There are, however, many alternative channels of blood supply, as demonstrated in two of his cases in which lesion of the specific artery of the node and bundle failed to give any electrocardiographic evidence of a lesion. The article is well illustrated.

N. ENZER.

STORAGE OF IRON FOLLOWING ITS ORAL AND SUBCUTANEOUS ADMINISTRATION. C. J. POLSON, Quart. J. Med. **23**:77, 1929.

Ten cubic centimeters of undiluted dialyzed iron containing 0.5 Gm. of iron was administered orally and subcutaneously in daily doses to rabbits. The experiments lasted from one hundred and seventy-two to four hundred and fifty days. The rabbits receiving the iron orally showed a maximum storage in the liver. There was no increase of iron in the lungs. The kidneys in some cases showed excessive iron. The chemical analysis of the subcutaneous series also showed high concentration in the liver, none in the lungs, an excess in both the kidney and spleen, and also a considerable amount in the dried subcutaneous tissue. The liver lobules contained iron diffusely. Coarse granules could be seen in the liver cells around the nuclei, generally at the outer third of the hepatic lobule. A few Kupffer cells contained iron. Iron could be stained in the spleen. There was an excess in the cecum and kidney, no excess in the bone-marrow and none in the lungs. In the subcutaneous series, the liver contained numerous giant cells, and the concentration of iron was much greater than in the oral series, although it appeared later. There were no giant cells noted in the oral series. There was more iron in the spleen after oral administration than after intravenous administration. The high concentration of iron in the cecum and kidney is due to excretion.

N. ENZER.

THE REACTION OF TISSUES TO THE ASBESTOS FIBER, WITH REFERENCE TO PULMONARY ASBESTOSIS. S. ROODHOUSE GLOYNE, Tubercle **11**:151, 1930.

Asbestos fibers, when injected into the body, act as a benign irritant producing granulation tissue with many asbestosis giant cells, presumably an attempt to destroy the asbestos fibers by phagocytosis. Connective tissue is formed in due course, but the giant cells persist. The asbestosis giant cell is readily distinguished from the tuberculosis giant cell. Asbestosis bodies are not found. Asbestos injected repeatedly intravenously appeared to have no toxic effects on distant tissues.

H. J. CORPER.

PULMONARY ASBESTOSIS: DEATH FROM TUBERCULOSIS TWO YEARS AFTER FIRST EXPOSURE TO THE DUST. W. BURTON WOOD and D. S. PAGE, Tubercle **11**:157, 1930.

The rapid evolution of the tuberculous process in a patient who had been exposed to asbestos dust was a feature of a case here reported. A large number of asbestosis bodies was present in the lungs two years after the first exposure to the dust.

H. J. CORPER.

BLOOD CONTENT OF THE HUMAN SPLEEN. H. HARTWIG, Beitr. z. path. Anat. u. z. allg. Path. **83**:431, 1929.

To determine the blood content of the spleen, the organ, carefully isolated at necropsy, was perfused with water until the perfusate was colorless. The hemoglobin content of the blood of the right side of the heart and of the perfusate having been determined, it was possible to calculate the quantity of blood represented by the hemoglobin of the perfusate. A pressure of from 170 to 180 mm. of mercury was used for the perfusion, the time required varying from one and one-half to six hours. The quantity of perfusing fluid varied from 6.5 to 70.5 liters, the average being 20.5 liters. One hundred and fifty-two spleens, obtained in the course of 194 necropsies on persons ranging in age from 5 to 86 years, were found satisfactory for perfusion. Of the perfused spleens, only those in which microscopic examination after perfusion revealed no red corpuscles in the tissue were used for the final calculation of results. Hartwig's figures are therefore based on sixty-seven spleens. The normal spleen with an average postmortem weight of 169 Gm. was found to have a blood content of 56 cc. Blood constituted 33 per cent of the postmortem weight of the normal spleen. The lowest figures were obtained for the spleens from cases of generalized anemia, for which group the average weight was 115 Gm., of which blood constituted 22 per cent. It may be noted that the printed tabulation contains a misprint of this figure, which is given as 59. The highest figures were obtained for passively congested spleens. The average weight of the spleens from this group was 249 Gm., the blood content being 116 cc., or 49 per cent of the total weight. The blood content of the spleen is estimated at 1.3 per cent of the total blood for the normal spleen, 0.7 per cent for the spleen from cases of generalized anemia, and 2.6 per cent for the spleen passively congested. These values are much lower than those obtained by other methods for the spleens of living animals, in which the spleen is held to act as an important reservoir of the splanchnic blood. The human spleen is probably smaller after death than at any time during life. Hartwig estimates the decrease at from one-fifth to one-third. To determine the maximum fluid capacity of the spleen, the organ, after completion of the perfusion, was filled with water to its maximum capacity at a pressure of 180 mm. of mercury. By this method the average maximum fluid capacity of the normal spleen was found to be 202 cc., as compared with an average postmortem blood content of 56 cc. For the spleen from cases of generalized anemia, the average maximum capacity was 104 cc. and for the passively congested spleen, 230 cc. The determinations of the maximum capacities gave considerable deviations from the average for each group. Hartwig estimates the blood content of the normal spleen during life at from 50 to 200 cc. and believes that the figure lies in the upper half of this range.

O. T. SCHULTZ.

ACQUIRED PATHOLOGIC FISSURES OF THE BRAIN. MARIA MITTELBACH, Beitr. z. path. Anat. u. z. allg. Path. **83**:445, 1929.

The author presents a description of five examples of a lesion of the brain that she believes has not been previously described, except for a brief report of one of the cases of her series made by Spatz. The lesion is a characteristic fissuring of the convolutions of the brain. The fissures are narrow, slitlike and long,

and are situated in the middle of the convolutions, following the windings of the latter. Short lateral branches are given off from some of the main fissures. The pia may run smoothly over the fissures, so that the latter would be overlooked if the pia is not removed. A loose network of connective tissue dips down into some of the fissures from the undersurface of the pia. The convolutions of the convex surface of the frontal and anterior third of the parietal lobes were involved, the distribution being symmetrical in the two hemispheres. Macroscopically, on cross-section the fissure appears as a narrow funnel-shaped defect that does not extend entirely through the cortex. Microscopically, the cell layers of the cortex extend in regular order to the margins of the defect. At the bottom of the latter is a narrow glial scar in which both glia cells and fibers are increased. In this area the regular arrangement of cells and fibers is disturbed. In preparations stained for myelin sheaths, the scar reveals a dense network of irregularly entwined myelin sheaths in which neurofibrils can be demonstrated. This histologic picture is identical with that of the plaques fibromyeliniques described by C. and O. Vogt. No remains of the lost cerebral substance were present in any of the lesions, which leads to the conclusion that the lesion is an end-stage of a process, the earlier stages of which have not been seen. The fissures can be readily distinguished from congenital microgyria, from the cortical defects due to arteriosclerosis of the pial vessels, and from the granular atrophy due to sclerosis of the finer vessels of the cerebral cortex. The lesion bears a close resemblance to the defects seen in the base of the brain as the result of concussion. Although there was no history of trauma in the cases reported, the author believes trauma to be the most plausible explanation of the pathogenesis of the fissures. In none of the cases were there symptoms that could be ascribed to the lesion described.

O. T. SCHULTZ.

BACTERIAL LOCALIZATION IN AREAS OF CEREBRAL SOFTENING. F. QUEDNAU, Beitr. z. path. Anat. u. z. allg. Path. **83**:471, 1929.

In two cases, what appeared to be multiple abscesses of the brain proved on closer examination to be areas of cerebral softening in which secondary localization of bacteria and suppuration had occurred. In one case the cerebral infarction was due to arteriosclerosis, and in the other to syphilitic arteritis. Each patient had suffered shortly before death with a pneumococcic infection of the lung. Pneumococci were cultivated from the blood stream and were seen in sections of the cerebral lesions. In one case streptococci were also present in the lesions of the brain. In experiments on animals, aseptic injury of the brain was produced by puncturing the skull of rats with a scalpel. On the second or third day after the trauma, bacteria were injected intravenously. In three animals that received staphylococci and in three that received hemolytic streptococci, no infection of the traumatized brain tissue occurred. In one rat that received pneumococci, suppuration of the injured tissue combined with purulent meningitis led to the death of the animal. Quednau believes that brain tissue that has undergone softening, either as the result of injury or of infarction, is a focus of lessened resistance and a site of election for the localization of any bacteria which may be present in the blood stream, especially pneumococci. He thinks that some of the idiopathic abscesses of the brain and abscesses following trauma to the head but in which no connection between the abscess and injury of the skull can be detected may be due to secondary infection of injured brain tissue.

O. T. SCHULTZ.

HEALING OF THE INJURED ARTERIAL WALL. A. SSOLOWJEW, Beitr. z. path. Anat. u. z. allg. Path. **83**:485, 1929.

Much of the literature on the reparative phenomena of the arterial wall relate to the changes that follow section and suture of arteries, or ligation or crushing. In the author's experiments, a procedure was used that did not destroy the elastic tissue, namely, cauterization of the outer surface of the vessel by means of a

red-hot wire. The rabbit was used, and the common carotid arteries and the abdominal aorta above the bifurcation were selected for study. The vessels were removed for microscopic examination at varying intervals ranging from two to ninety days after the cauterization. In the early stages, there is slight polymorphonuclear infiltration of the inner and middle zones of the media and a few polyblasts may be present. The author thinks that these cells wander into the tissue from the vessel lumen. If the elastic tissue is not destroyed, the reparative phenomena occur chiefly in the media. The muscle cells of the media at the margin of the injured area proliferate, and the young cells grow along the surface of the layer of elastic fibers. The latter form a compressed and compact layer as the result of the disappearance of original tissue elements from between the fibers. The regeneration of the media may be so complete that the site of the injury is difficult to detect. If the elastic fibers form such a compact layer that the regenerating muscle cells cannot penetrate between the fibers, a latter replacement of mucle cells by connective tissue occurs. If the injury was great enough to cause destruction of the elastic tissue, regeneration of the media does not occur, but the injury is repaired by an ingrowth of fibroblastic tissue from the adventitia. The intima may be thickened in the injured area. In early stages, proliferation of endothelium is seen, the endothelial cells forming a layer several cells thick. Although the author grants the formation of muscle and connective tissue of the intima from the proliferated endothelium, he saw no evidence of such transformation in his material. If the elastic layer was not too compact, young muscle cells could be seen to make their way between the fibers to take part in the regeneration of the intima. A new formation of elastic fibrils could not be detected in the media, but was seen in the adventitia. The process of healing of the injured arterial wall is a very slow one and requires a long time for its completion.

O. T. SCHULTZ.

THE CELL REACTION IN LUPUS. K. A. HEIBERG, Virchows Arch. f. path. Anat. **272**:375, 1929.

Only in 41 of 100 specimens of lupus were giant cells found and in some of these only few. A large number of plasma cells were found in 2, a considerable number in 5. The absence of central necrosis may be due to rapid absorption as well as to lack of its formation. The relative amount of epithelioid cells, lymphoid cells and plasma cells and their distribution mainly depends on the age of the lesion and on treatment.

ALFRED PLAUT.

SYSTEMIC RETICULOSIS. O. BYKOWA, Virchows Arch. f. path. Anat. **273**:255, 1929.

An acute infectious disease probably influenza, together with signs of decompensation, brought an obese woman 69 years of age to the hospital. She looked anemic and cyanotic. Enlarged lymph nodes varying in size up to that of a walnut and even a plum were felt in the axilla and groin. They were hard, isolated from each other, but not tender. There were 4 per cent eosinophils, 65 per cent lymphocytes and 14.5 per cent monocytes. The number of neutrophils decreased, and the monocytes increased to 25 per cent; they were the only form, the absolute figure of which was never below the normal.

At autopsy, an enlargement of spleen and lymph nodes was found. The lymph nodes were soft, the marrow in the long bones was red and the liver was not enlarged. In the lymph nodes many clear cells prevailed which had phagocytic properties. In addition, there were round basophil cells with large, round nucleus and many nucleoli. The architecture of the lymph node was destroyed, the cells closely enmeshed in a system of fibrils. The spleen gave a corresponding picture. In the bone-marrow many of these cells contained erythrocytes and pyknotic nuclei. Very few granulocytes were left. In the tonsils also the lymphatic tissue was atrophic. Reticulum cells and basophil cells occupied the whole organ and were found in the neighboring muscle also. Smaller accumulations of such cells

were found in other organs. The oxidase reaction was negative. These results indicated a systemic overgrowth of reticulum cells with atrophy of lymphatic parenchyma. In the bone-marrow the erythroblastic system was less damaged than the remainder. The disease is regarded as an aleukemic reticulosis. The cause is unknown, as it is in the other forms of reticulo-endotheliosis.

ALFRED PLAUT.

THE HILUS CELLS OF THE OVARY. H. O. NEUMANN, Virchows Arch. f. path. Anat. **273**:511, 1929.

Negative chrome reaction does not preclude the sympathetic nature of the hilus cells. When one kills mice with ether and chloroform, even serial sections do not lead to the detection of any chromaffin cells. In decapitated animals, with the same technic, the chrome reaction was negative. Guinea-pigs were killed with chloroform during labor after one of the young was born. In the young that was born before the administration of chloroform to the mother, the chrome reaction was positive; in the others it was negative.

Cells similar to the hilus cells of the ovary are found in the hilus of intestine. All these cells are special sympathetic elements which sometimes exhibit more of a paraganglionic character, sometimes more of the character of cells with internal secretion.

ALFRED PLAUT.

AGGREGATION OF A SPECIAL CELL IN THE MALE GENITALS OF ANIMALS. ZENICHI SHIOSAWA, Virchows Arch. f. path. Anat. **273**:531, 1929.

The cellular mass was found in four of five rabbit testicles when serial sections were made. In representative sections they were found in 80 of 287 specimens. They were absent in dogs. The location was either the septum or the neighborhood of the rete or the connective tissue of the coni vasculosi. They occurred with equal frequency in healthy and in diseased rabbits. The size varied from 45 to 1,100 microns, their number in one testicle from one to ten. The organ is surrounded and occasionally subdivided by connective tissue. The cells are polyhedral, round or ovoid, with much cytoplasm which may be granular or vacuolized. The cells measure from 7 to 10 microns the nuclei from 4 to 7 microns; they are vesicular, clear, with one or two nucleoli. Oxydase, chromaffin substance, epinephrine, Altmann granules, glycogen and fat are absent. The cells change rapidly after death. They store neither carmine nor india ink. The organ can be damaged by feeding the animals hydrous wool fat, by exposing the scrotum to sunlight, and by deficient diet. It seems to be more resistant than the testicle. In spite of the relatively rare occurrence (28 per cent), it is considered a normal organ. Its function is unknown.

ALFRED PLAUT.

EPENDYMAL EMBRYONIC NEUROGLIOMA OF PINEAL GLAND. G. L. DERMAN and M. A. KOPELOWITSCH, Virchows Arch. f. path. Anat. **273**:657, 1929.

The tumor (2.75 by 2 by 2 cm.) springs from the commissura habenularum. A groove separates a small posterior portion which is the pineal gland. Characteristic rosets are found in the tumor. The clinical symptoms appeared during pregnancy, receded after delivery, and reappeared with increased intensity in the second month of the following pregnancy. About eighty cases of primary tumor of the pineal gland are reported in the literature.

ALFRED PLAUT.

SOLID TERATOMA AND MEDULLARY OSTEOCHONDROMA OF CORPUS UTERI. W. MANN, Virchows Arch. f. path. Anat. **273**:663, 1929.

The literature contains no well established report of intra-uterine teratoma. A healthy woman, aged 31 had a prolonged menstruation and, two months later, pain at the onset of menstruation. The uterus was enlarged, and in the distended cervix a firm, strawberry-like tumor was felt which was diagnosed as a submucous

myoma but proved to be a teratoid tumor. Two months after its removal, bleeding and pain led to hysterectomy.

The left tubal angle was the seat of a tumor the size of a small apple. It was partly nodular and partly polypoid; the consistency varied. There were cysts with different contents. The outline against the myometrium was indistinct. Microscopically, skin, hairs, glands, bone and cartilage, etc., were found. All three germinal layers were represented; most tissues had embryonic character. Mesoderm and entoderm prevailed. This tumor could not possibly have originated from an ovum of the patient. The ovulogenous theory of teratomas probably is erroneous. One must, for this uterine teratoma, refer to one form of the blastomeric theory. It is unknown why in this instance a solid teratoma had formed and not a dermoid cyst.

The second tumor described in this paper was found in a tripara 38 years of age, who had had one breast amputated twelve years before. She had a squamous cell carcinoma of the cervix and was pregnant. After an unexpected premature labor she had fever for many weeks. She received radiation treatment. The cervical tumor was a squamous cell carcinoma without hornification.

In the right tubal angle an osteochondroma the size of a walnut was situated within the myometrium. It was surrounded by a thin layer of firm connective tissue. The cartilage was hyalin. The ossification partly looked similar to an epiphyseal line. The marrow contained few cells; there was no blood formation. Germinal misplacement seemed the best explanation.

ALFRED PLAUT.

PHYSIOLOGY OF CELLULAR RESPIRATION IN ITS RELATION TO NEW HISTOLOGIC OBSERVATIONS ON LEUKOCYTES AND HEART MUSCLE. E. SEHRT, Virchows Arch. f. path. Anat. **273**:701, 1929.

Histologic methods that can demonstrate single phases only, not processes, on the other hand, are not subjected to many of the sources of error that may interfere with physiologic work. The granules that form a permanent part in the structure of the living cell obviously must be centers of cellular respiration. They are the seat of the oxydase reaction, stabile and labile. The functional iron of the cell so far has escaped histochemical demonstration except for Katsunuma's observations in the vaginal muscle. The lipoid nature of the oxydase granules often has been suggested but never has been proved. These granules are identical with the fine granules that one can see under oil immersion in unstained sections of unfixed material. Since the beginning of histology (Koelliker), the lipoid character of these granules has been suspected, but never has been proved. By means of a modified double sudan stain, Sehrt has succeeded in staining granules of the myeloic cells red with sudan. In the polymorphonuclear leukocytes, the protoplasm is full of dark red granules, which, in distribution, shape and size, are identical with the common neutrophil granulation. The eosinophil granulations are very distinct. The majority of the transitional cells contain red granules which, in size, shape and color, are similar to those of the polymorphonuclear cells. They are mainly located at the indentation of the nucleus. Thirty-nine per cent of the transitional cells are free of granules. Basophil cells show their coarse granules in dark red color, but in addition there are smaller granules, as in the neutrophil leukocyte. In normal people, all large mononuclear cells contain many red granules, just as the neutrophil cells do. The lymphocytes are entirely free of sudan stained granulations. The granules are insoluble in cold alcohol, cold acetone, boiling acetone, cold ether, cold benzine, cold chloroform; they are easily soluble in xylol, in hot absolute alcohol; they slightly dissolve in acetone when left there for a long time.

Thus, oxydase granules and these sudanophil granules are identical. They are morphologically alike. Number and distribution of both kinds of granules in the neutrophil leukocytes are identical. The four groups into which Naegeli divides the neutrophil cells, according to their oxydase granulations, can be established for the sudanophil granulations as well.

The labile oxydase granules contain lipoid also. For their demonstration the author used the following method. Frozen sections of unfixed material are spread on the slide and allowed to dry. These sections practically never come off the slide and can be used for nearly all staining methods. They are not torn, and they do not shrink. They do not contain traces of water; they have all the advantages of the paraffin or the frozen section. Another modified sudan method is used for the labile oxydase granulation. The whole heart muscle appears studded with the fine red granules, which follow not only the lengthwise striation, but the cross-striation also. Connective tissue, blood vessels, etc., are entirely free of granules. After exposure to fat solvents, the sudan stain is negative.

These granules adsorb fatty acids. If one puts the dry frozen section into liquid animal fat at a temperature of 40 C. and removes the fat mechanically, the granules will stain with any sudan method. This experiment becomes negative when dry paraffin section has been in a fat solvent for a few days. These observations are in accord with Warburg's physiologic experiments. These granules are soluble in absolute alcohol, xylol, ether, chloroform, acetone and benzine. They obviously consist of mono-amino-phosphatids. The same substances have been found by Meyerhof in ether-alcoholic extracts of heart muscle. The granules of the myeloic cells, on the other hand, consist of saturated phosphatids and cerebrosids.

Cellular respiration and oxydase reaction are dependent on lipoids. The functional iron in the cell must be located on the granules. Every granule is an oxidation center with active surface.

ALFRED PLAUT.

CIRCUMSCRIBED LIPOID DEPOSITS IN THE MUCOSA OF THE STOMACH AND INTESTINE. FRIEDRICH FEYRTER, Virchows Arch. f. path. Anat. **273**:736, 1929.

In 1,300 autopsies, circumscribed lipoid deposits were found twenty-five times in the mucosa of the stomach. They are found in between 3 and 10 per cent of persons over 45, depending on the occurrence of atherosclerosis, cholelithiasis and obesity. The season seems to play a rôle also. Generally only one island of lipoid cells was found. They are easily overlooked in the routine slide. It is impossible to say why the lipoid deposits are circumscribed and isolated. In the intestine, the lipoid deposits are a little different. Only twice in the 1,300 autopsies could they be seen with the naked eye, and in both instances in the duodenum. In the intestine, overgrowth of the lipoid cells is found. It is absent in the stomach. There is no indication of a congenital anomaly at the point of these deposits.

ALFRED PLAUT.

FOUR CASES OF CONGENITAL OCCLUSION OF INTESTINE. HANS NAHRATH, Virchows Arch. f. path. Anat. **273**:747, 1929.

The fact that between multiple stenoses of small intestine, meconium and lanugo could be found, leads to the conclusion that in this one instance the cause of the occlusion must have come from within the intestine. The outer layers and mesenteric vessels were found completely intact, without any angulation or torsion. The time when the liver begins to secrete bile is much later than the time when parts of the intestine pass through a solid stage. Thus, fetal enteritis is given as a cause for this case of congenital multiple stenoses of small intestine. In the three other cases described, disorders in the obliteration and separation of the omphalomesenteric duct had been leading to meconium peritonitis.

ALFRED PLAUT.

HYPOPLASIA OF CORPUS CALLOSUM IN NEW-BORN INFANTS. HEINRICH V. HAYEK, Virchows Arch. f. path. Anat. **273**:767, 1929.

The corpus callosum was half the normal size. The brain had been fixed in situ. The gyrus cinguli was interrupted and thin, corresponding to the absence of corpus callosum fibers. There were no other important anomalies.

ALFRED PLAUT.

THE LACUNAR RESORPTION OF UNCALCIFIED DENTINE. W. BAUER, Virchows Arch. f. path. Anat. **273**:780, 1929.

In the teeth as well as in the bone, the hard substances are entirely passive in the processes of bone formation and of bone destruction. Both processes depend entirely on the condition of the surrounding soft parts, notably the blood circulation.

ALFRED PLAUT.

TRAUMATIC INFLAMMATION INFLUENCED BY X-RAYS. SHUICHI FUKASE, Virchows Arch. f. path. Anat. **273**:794, 1929.

Radiation of an incision in the abdominal skin of a rabbit immediately after making the incision leads to rapid healing. Exudation and infiltration are less in the radiated part of the wound. In the radiated part, round and spindle-shaped cells with oxyphil granulation are found, cells which are not present in the circulation of the rabbit. The author believes that these cells originated in loco. He thinks that oxyphil cells which are formed from connective tissue cells generally develop segmentation of the nucleus at the same time, while in his experiment the radiation prevented the segmentation of the nucleus.

ALFRED PLAUT.

THE ACTION OF LIVER DIET ON PERNICIOUS ANEMIA. T. FAHR, Virchows Arch. f. path. Anat. **273**:864, 1929.

Fahr starts from the question why the liver of the patient with pernicious anemia should be unable to furnish the substance which is active in the liver extract or other liver preparation administered by mouth. In the livers of fifty patients who died of pernicious anemia, the tissue changes were unimportant, irregular, and showed no relation to the degree of the anemia. Great liver destruction, such as occurs in acute yellow atrophy, does not lead to anemia; the red cell count generally is high, and it is doubtful how far the high figures in liver atrophy are due to inspissation of the blood. Dogs were fed with raw liver alone, spleen alone, or portal lymph nodes alone. The results were quite ambiguous, and the experiments therefore were discontinued. These experiments with dogs do not prove much concerning anemia in man, since the iron metabolism in dog and man is quite different. Dogs normally show much erythrophagia in the portal lymph nodes. In the human portal lymph nodes there is little erythrophagia. It becomes much more marked in anemia, never as marked as it is normally in the dog. In this connection, Fahr quotes the fact that the blood from patients with anemia inhibits the development of plant germs and that dog blood does that normally. Liver therapy in two normal young physicians resulted in a slight increase of red cells and hemoglobin. When there is severe damage done to the bone-marrow, as in osteoplastic anemia, the liver diet is ineffectual. No relation could be found between the paucity of iron in the spleen and the degree of anemia. For the question, how the clinical picture of anemia can be explained by some toxic agent, the funicular myelitis is of great importance. There are patients with marked funicular myelitis and relatively little anemia. In rare instances a patient dies of the myelitis, and the blood picture, and the autopsy findings are hardly sufficient for a diagnosis of pernicious anemia. The anemia itself cannot be due to a toxic factor, and the liver diet probably acts as a substitution therapy in a similar way as vitamin feeding works in scurvy.

ALFRED PLAUT.

OSTEITIS DEFORMANS (PAGET). ERNST FREUND, Virchows Arch. f. path. Anat. **274**:1, 1929.

Several problems were studied in sections from the femur of a 71 year old woman who had been in bed for eight years after fracture of the neck of femur. The femur partly was normal, and the beginning phases of Paget's disease could be studied. In the process of destruction of the old tela ossea, fibrillar marrow

hollows out the old trabeculae, between which, however, the old fatty marrow remains. Under the periosteum, a layer with thin, mostly noncalcified trabeculae of reticular bone is situated; the author calls it the parosteal layer. The Paget bone becomes thicker by a kind of parosteal callous formed in the parosteal layer. No osteoblasts participate in this process. There were changes in the joint cartilage which did not completely correspond to the picture of deforming arthritis.

ALFRED PLAUT.

OSTEOGENESIS IMPERFECTA AND ENDOCRINE SYSTEM. ERIK JOHANNES KRAUS, Virchows Arch. f. path. Anat. **274**:37, 1929.

In two instances of osteogenesis imperfecta no signs of premature development of the endocrine glands could be found.

ALFRED PLAUT.

SYSTEMIC ANGIOPLASTIC SARCOMA IN SPLEEN, LIVER, AND BONE-MARROW. WALTHER SCHLOPSNIES, Virchows Arch. f. path. Anat. **274**:85, 1929.

The tumor in its main features corresponds to the hemangio-endothelioma as described by B. Fischer-Wasels. The patient was a woman 38 years old; her previous history was irrelevant. After her second delivery about three months before admission she felt tired, had no appetite, and complained of swelling of the legs. She became progressively weaker, was pale, but had no pain. An area of massive dulness occupied the left lower part of the thorax; the whole left side of the abdomen was occupied by the enlarged spleen. The long bones and the sternum were tender on percussion. The blood contained many normoblasts; among the 7,000 leukocytes, 5 per cent were myelocytes, 5 per cent young forms.

The enlarged spleen (2,600 Gm.) was fixed by adhesions; it was firm; the cut surface was irregularly gray and red, with many hemorrhages and some reddish gray, wedgeshaped foci under the capsule. The bone-marrow was red throughout. Many round, cyanotic foci were found in the bone-marrow. The liver also contained countless, mostly round, well outlined nodules, the smallest ones hardly visible, the largest ones the size of a walnut.

Microscopically, the tumors consisted of spindle-shaped elements with very dark, large nuclei. These cells formed blood vessel-like spaces and in other areas accumulated into sarcoma-like masses. All gradations, from the normal endothelial cell to the tumor cell, could be seen. Single large tumor cells were found in otherwise normal capillaries. In the spleen, most of the normal structure was destroyed. There were large areas of necroses, the tumor was mostly sarcoma-like. Mitotic figures were not very frequent. Large blood spaces were formed by the tumor cells; their walls were free from elastic fibers. Occasionally, small foci of blood formation were seen.

In the bone-marrow, the picture was different. Very little normal bone-marrow was found. The tumor, which had replaced the bone-marrow, mainly consisted of thinner spindle cells, partly with wider blood spaces.

This tumor cannot be explained by metastasis from a single primary focus. There was a diffuse, tumorous change of the endothelium in liver, spleen and bone-marrow. The other organs were free. There are cases in the literature in which other organs, like the thyroid, for instance, show the same tumor. The cause of the tumor is unknown; one has to assume a disturbance in the embryonic "Anlage" of the endothelial system. This is a disease of the endothelial cells alone. The reticulum cells are not involved.

ALFRED PLAUT.

AMYLOIDOSIS OF KIDNEY IN CATTLE. T. PRIMGAARD, Virchows Arch. f. path. Anat. **274**:111, 1929.

The so-called parenchymatous chronic nephritis in cattle is primarily an amyloidosis of kidney complicated by interstitial and parenchymatous inflammation.

In a few instances, amyloidosis could be produced in mice and rats by injection with bacteria cultivated from the cattle, but one animal only showed amyloidosis of the kidneys.

ALFRED PLAUT.

ISOLATED AMYLOIDOSIS OF SEMINAL VESICLES. O. LUBARSCH, Virchows Arch. f. path. Anat. **274**:139, 1929.

General amyloidosis seldom reveals amyloid deposits in the seminal vesicles. The amyloid then is found mostly in the walls of blood vessels. In the few instances of isolated amyloidosis of seminal vesicles, the deposits were situated between epithelium and propria, as found in a man 49 years old who died of lymphatic leukemia. No explanation of this isolated amyloidosis can be given.

ALFRED PLAUT.

CALCIFICATION OF THE MYOCARDIUM IN DOMESTIC ANIMALS. W. S. TSCHERNIAK and S. VORONZOV, Virchows Arch. f. path. Anat. **274**:154, 1929.

Calcification of myocardium, mostly in the papillary muscles of the left side of the heart, is not rare in horses and dogs. The inflammatory reaction obviously is secondary to the calcification. It is interesting that in four dogs which died of distemper, such calcification was found.

ALFRED PLAUT.

NUTRIENT VESSELS OF AORTA. H. SMETANA, Virchows Arch. f. path. Anat. **274**:170, 1929.

The distribution of nutrient vessels of aorta was studied by injection of the whole thorax. Tandler's cold gelatin solution with prussian blue was used. A detailed description of the vessels is given, illustrated with thirteen drawings. Anastomoses of larger arterial branches are rare. One generally can distinguish three portions of the thoracic aorta: first, the lower half of the aorta ascendens, which receives its blood from the coronary arteries; second, the portion up to the origin of the left subclavian artery; and third, the descending portion. The distribution of the syphilitic aortitis cannot be sufficiently explained by the distribution of the vasa vasorum. The fact that a disease which has such a different cause as, for instance, the filaria aortitis of the dog, has the same distribution, speaks against any important rôle of the vasa vasorum, especially since the distribution of the vessels in the dog is quite different from that in man.

ALFRED PLAUT.

Pathologic Chemistry and Physics

THE RELATION OF PARTICLE SIZE TO MECHANISM OF DYE EXCRETION BY THE KIDNEY. J. OLIVER and E. SHEVKY, Am. J. Physiol. **93**:363, 1930.

There is a correlation between the degree of filtrability of a series of dyes and the mechanism of their elimination as studied in the perfused kidney of the frog. Easily filtrable dyes, of small particulate size, pass readily through the glomerular membrane; those of moderate size are discharged by tubular activity, and those of large size cannot be eliminated.

H. E. EGGERS.

IS IT POSSIBLE TO PRODUCE PERMANENT CHANGE IN THE ACID BASE BALANCE? M. MECKLENBURG, Beitr. z. Klin. d. Tuberk. **73**:232, 1929.

No clinical or experimental method is known at the present time by which a permanent change in the acid base balance can be produced without endangering the life of the organism.

MAX PINNER.

THE DIFFERENTIATION OF THYROXIN IODINE FROM INORGANIC IODINE BY THE MEMBRANES OF THE LIVING ORGANISM. W. LIPSCHITZ, Klin. Wchnschr. **9**:642, 1930.

Dogs endured large single doses (20 mg. per kilogram of weight) of thyroxin intravenously; most of it was found in the plasma. The initial concentration (from 5 to 6 per cent I) decreased within one or two days to less than one-tenth without appreciable amounts passing into the urine. Little, if any, of the circulating thyroxin is secreted by the stomach and the parotid. Small amounts are split into iodides, which then appear in gastric and parotid secretions. The gland membranes of the living organism fractionate the various iodine compounds in the blood so that a determination of the iodine content of the gastric juice or the parotid secretion enables the approximation of the circulating iodide of the blood. It approaches about one-tenth and may be more closely determined by testing in each individual the concentrating power of the glands, following the intravenous injection of small amounts of iodide.

EDWIN F. HIRSCH.

CHEMOTAXIS OF LEUKOCYTES. C. HÄBLER and C. WEBER, Klin. Wchnschr. **9**:760, 1930.

Experiments by the Pfeiffer method demonstrated that chemotaxis parallels the surface tension activity of the test solution and that changes of the actual reactions and of the osmotic tension have no effect. No specific action of the cations of the alkali and earth metals was demonstrated. The differences in chemotaxis of various organic acids disappears when their solutions are brought to the same surface tension.

EDWIN F. HIRSCH.

PHENOL IN THE BLOOD IN CIRRHOSIS OF THE LIVER. E. BECHER, München. med. Wchnschr. **77**:751, 1930.

Moderate increase of phenol in the blood and especially the presence of free phenol in the blood occur in many cases of cirrhosis of the liver. These results suggest a disturbance in the detoxication of intestinal products in cirrhosis of the liver.

AUTHOR'S SUMMARY.

CHOLESTEROL CONTENT OF BLOOD AND BLOOD SERUM IN PULMONARY TUBERCULOSIS. F. WARNECKE, Ztschr. f. Tuberk. **56**:137, 1930.

In minimal and moderately advanced tuberculosis, the cholesterol content of the blood is found within normal limits. In far advanced and toxic conditions, the cholesterol is decreased. The decrease in cholesterol is not specific for tuberculosis, but occurs in all infectious diseases that produce cachexia.

MAX PINNER.

Microbiology and Parasitology

INTRANUCLEAR INCLUSIONS IN YELLOW FEVER. E. V. COWDRY and S. F. KITCHEN, Am. J. Hyg. **11**:227, 1930.

Cytologic studies on the intranuclear inclusions in experimental yellow fever in monkeys and in human beings would seem to show that the nuclear response in yellow fever is of the same general type, though different in detail, as that which occurs in many other virus diseases, especially chickenpox, herpes, virus III disease and submaxillary disease.

JOHN PHAIR.

THE LOCATION OF YELLOW FEVER VIRUS IN INFECTED MOSQUITOES AND THE POSSIBILITY OF HEREDITARY TRANSMISSION. NELSON C. DAVIS and RAYMOND C. SHANNON, Am. J. Hyg. **11**:335, 1930.

Yellow fever virus has been demonstrated in the head, thorax and abdomen of mosquitoes before the bites are infective. Transmission has been secured by the inoculation of legs, ovaries, salivary glands, midguts and hindguts of infected

Aedes aegypti. No transmission has been obtained from the inoculation of hemocelic fluid or of mouth parts of infected mosquitoes. Virus is occasionally present in the dejecta from infected mosquitoes. No definite transmission has been secured from the inoculation of eggs laid by infected mosquitoes. No evidence has been obtained that virus ever passes from one generation of mosquitoes to the next through the eggs (hereditary transmission). No transmission has been accomplished by adults bred from larvae which had consumed large numbers of infected mosquitoes.

AUTHORS' SUMMARY.

AN EPIDEMIC OF INFLUENZA IN AN ISOLATED COMMUNITY—NORTHWEST RIVER, LABRADOR. W. G. SMILLIE, Am. J. Hyg. **11**:392, 1930.

An epidemic of influenza occurred in Northwest River, an isolated community in Labrador, in 1928. The date of introduction of the disease into the community was known and it was possible to trace the epidemic throughout its course. It seems probable that the virulence of the infective agent remained fixed, and the dosage was uniform. There occurred, however, a marked variation in resistance of various individuals to infection. It seems probable that this resistance was nonspecific. The infective agent disappeared completely from the community in a short time, for a presumably highly susceptible group of Indians entered the community one month after the last case had occurred and none of them was affected.

AUTHOR'S SUMMARY.

PARATYPHOID—ENTERITIDIS MENINGITIS. FRANK B. LYNCH, JR., and SAMUEL A. SHELBURNE, Am. J. M. Sc. **179**:411, 1930.

A review of the literature reveals fifteen previous cases of meningitis from which bacilli of the *paratyphoid-enteritidis* group were isolated, and a sixteenth case is here reported. Most of the cases occurred in children. All the patients aged a year or less died, including the author's patient. In most of the cases the fluid was purulent, or showed a predominance of polymorphonuclear neutrophils. In all the previously reported cases in which blood culture was made, it was positive. In our case postmortem blood culture was negative.

AUTHORS' SUMMARY.

TRICHOMONAS VAGINALIS. C. H. DAVIS, Am. J. Obst. & Gynec. **18**:575, 1929.

Davis finds that these organisms may be killed when exposed in a water bath to 46 C. for ten minutes. They likewise are destroyed by exposure to cold (9 C.) for twelve hours.

A. J. KOBAK.

LESIONS OF FOWL-POX AND VACCINIA. C. EUGENE WOODRUFF, Am. J. Path. **6**:169, 1930.

The histology of lesions obtained in chick epithelium following inoculation with vaccinia virus alone, fowl-pox alone and the two viruses mixed is described. The characteristic virus bodies of fowl-pox, whether occurring in the skin or the cornea of the chick, give a positive reaction for fat. The Guarnieri bodies of vaccinia do not stain for fat. Ludford's statement regarding the identity of the virus bodies of vaccinia and fowl-pox in the chick is refuted. His observations are due, it is believed, to the utilization of tissue from a mixed lesion of fowl-pox and vaccinia.

AUTHOR'S SUMMARY.

TRANSMISSION OF DENGUE VIRUS FROM INFECTED TO NORMAL AEDES AEGYPTI. JOE H. ST. JOHN, JAMES STEVENS SIMMONS and FRANCOIS H. K. REYNOLDS, Am. J. Trop. Med. **10**:23, 1930.

It is possible to infect normal *A. aegypti* with dengue by feeding them through a guinea-pig's skin on a mixture of blood and macerated dengue-infected mos-

quitoes. Mosquitoes fed on this mixture later produced dengue fever in two human volunteers. This method should prove helpful in the study not only of dengue, but also of other virus diseases transmitted by insects.

AUTHORS' SUMMARY.

PARASITES IN THE BLOOD OF WILD MONKEYS OF PANAMA. HERBERT C. CLARK, Am. J. Trop. Med. **10**:25, 1930.

Ten varieties of monkeys are found in the Republic of Panama. Four of these are included in this survey, *Ateles geoffroyi, Cebus capucinis-imitator, Sairimi orstedii-orstedii* and *Alouatta palliata-inconsonans.* Tertian-like and quartan-like malarial parasites, microfilaria and trypanosomes were found in the blood of these monkeys. Autopsy of these monkeys revealed adult *Filaria* in the abdominal cavities of the red spider and white throated species, but no adults were found in the cavities of the Titi monkeys. An adult female monkey *(Macacus rhesus)* was inoculated with defibrinated blood of sixty monkeys, twenty-four of which showed signs of malarial infection. This female, however, presented no signs of illness, no elevation of temperature and no parasites in the blood for a period of six weeks following inoculations. No trypanosomes have been seen although she was inoculated with positive blood from three species. Guinea-pigs also failed to show trypanosome infection. The monkey should be a better animal than the bird for research work in malaria since it supplies a larger volume of blood, a type of red blood cell more closely resembling man's and species of *Plasmodii* that are difficult to distinguish from the benign species found in man. Some attempts at transfer of monkey malaria to man have failed in the Eastern hemisphere, yet it would seem proper to repeat this work in Panama if an infant monkey with an acute initial infection can be taken alive. Similar efforts with the trypanosome should be made with the horse.

JOHN PHAIR.

TUBERCULOUS INFECTION IN SCHOOL CHILDREN. E. FENGER, P. M. MATTILL and E. PHELAN, Am. Rev. Tuberc. **21**:183, 1930.

The incidence of tuberculous infection in school children in rural Hennepin County, Minnesota, is much lower than that usually reported. The "questionable positive" group may represent an infection that is of such low grade that it gives no appreciable reaction or it may represent a once active infection that is dying out. The markedly positive group represents those cases having received recent or repeated infections. The high incidence of tuberculous infection in contact cases is again shown. Malnutrition does not seem to predispose to infection nor does it necessarily follow after infection has taken place. X-ray plates are essential in determining pulmonary involvement in infected cases; there is not a complete correlation between the positive reactors and the x-ray film; nevertheless, the x-ray film gives much valuable information and should be taken in at least all of the positive reactors.

H. J. CORPER.

THE DISAPPEARANCE OF SCROFULA. H. R. M. LANDIS, Am. Rev. Tuberc. **21**: 195, 1930.

The author compares his observations of the incidence of scrofula at the end of the last century and at the present time and notes particularly that in the Episcopal Hospital in Philadelphia there were 136 cases of scrofula in 1900, while in 1928 there were 27 and only 8 of these occurred in children. In the Jefferson Medical College Hospital in 1900 there were fifty-four cases, while in 1928 only twenty were recorded and seven of these occurred in children. He cites numerous other statistics to indicate that both scrofula and tuberculous adenitis showed a decided decline.

H. J. CORPER.

FORTY STRAINS OF YEAST-LIKE FUNGI FROM SPUTUM. W. D. STOVALL and ANNA A. BUBOLZ, J. Infect. Dis. **45**:463, 1929.

Forty strains of yeastlike fungi isolated from sputum are reported in this study. They fall into four genera giving characteristic differences on culture mediums: *Monilia, Oidia, Endomyces* and *Saccharomyces. Monilias* comprise the largest group—thirty-seven strains. Of the others only one strain of each kind was encountered. All the organisms showed constant cultural reactions over a period of two years. On the basis of these observations, viz.—sugar fermentation and colony formation on malt agar in forty-eight hours at 37 C. incubation, we have been able to recognize three distinct types of *Monilia.* The results of animal inoculations were variable.

AUTHORS' SUMMARY.

EPIZOOTIC LYMPHADENITIS IN GUINEA-PIGS DUE TO AN ENCAPSULATED MUCOID HEMOLYTIC STREPTOCOCCUS. JOHN SUMTER CUNNINGHAM, J. Infect. Dis. **45**:474, 1929.

An epizootic of lymphadenitis affecting a small number of guinea-pigs has been described. The disease frequently terminated as a septicemia following superimposed experimental laboratory infections. The inciting organism in each case was an encapsulated hemolytic streptococcus of the B type, having large mucoid, mucilaginous colonies on blood agar. All strains of this streptococcus were culturally and serologically identical. In transfers from broth cultures and from cultures treated with a bacteriophage the colony changed to a small granular rough type. This dissociation of the strain occurred spontaneously and was apparently aided by the presence of bacteriophage. Serial lysis was not produced. Agglutinins for this streptococcus were found in high titer in a guinea-pig affected with typical "lumps," having large abscesses of the cervical nodes. Similar agglutinins were commonly present, though in lower titer, in the serum of normal rabbits. Rabbits given intravenous injections with washed (in salt solution), killed streptococci produced specific agglutinins in high titer. The titer was partially sustained for two months after the last injection. The lymphadenitis, although in a somewhat more acute form, was reproduced experimentally in guinea-pigs by subcutaneous injections of this streptococcus. The organism was highly virulent for rabbits and mice, killing these animals in from two to four days.

AUTHOR'S SUMMARY.

METABOLISM OF THE ABORTUS-MELITENSIS GROUP. JAMES G. MCALPHINE, WAYNE N. PLASTRIDGE and GEORGE D. BRIGHAM, J. Infect. Dis. **45**:485, 1929.

When grown on plain Fairchild peptone agar for several generations *Bacterium melitensis* and the porcine and human strains of *Bacterium abortus* tend to lose their ability to utilize dextrose. Growth on liver infusion agar, plain agar, nutrient broth or dextrose broth fails to restore completely this lost characteristic. Three transfers in liver infusion broth with continued incubation at 37 C. for two weeks caused these strains to develop a new form (mucoid) in which the dextrose-utilizing power was restored. The mucoid forms of the bovine strains tested did not utilize dextrose.

AUTHORS' SUMMARY.

VIBRIOTHRIX TONSILLARIS N. SP. THE ORGANISM OF ACTINOMYCES-LIKE TONSILLAR GRANULES. RUTH TUNNICLIFF and LEILA JACKSON, J. Infect. Dis. **46**:12, 1930.

From an actinomyces-like tonsillar granule a second strain of *Vibriothrix tonsillaris* has been isolated in pure culture, which produces roset and test tube brushlike forms similar to those seen in the original material. This strain is characterized by the production of masses of ovoid and irregular bodies and

filaments, which stain pink with Giemsa, from which bacilli and filaments appear to originate, and around which the stars and test tube brush forms seem to develop. This *Vibriothrix* produces lesions in the lung, bone and skin of rabbits and from the latter the organism was isolated in pure culture.

AUTHORS' SUMMARY.

THE TWO VIRUSES IN ENDEMIC TYPHUS (MEXICAN TABARDILLO). JOSE ZOZAYA, J. Infect. Dis. **46**:18, 1930.

The scrotal lesion produced in the guinea-pig, by the injection of endemic North American typhus, is not of typhus origin. The Rickettsia-like organisms found in the epithelial cells of the scrotal lesions in the guinea-pig have nothing to do with the production of typhus in the human being or in the animal. There are often two different viruses in the blood of patients with endemic typhus (typhus not transmitted by the louse), one the specific virus of typhus, and the other a virus pathogenic for the guinea-pig, producing in this animal scrotal lesions. The scrotal lesion-producing virus is transmissible, nonpathogenic for man, and has a short incubation period (two to four days) after being adapted to animal passage.

AUTHOR'S SUMMARY.

THE ACTION OF PANCREATIC JUICE ON BACTERIA. ALEXANDER A. DAY and WILLIAM M. GIBBS, J. Infect. Dis. **46**:26, 1930.

Pancreatic secretion obtained directly from the pancreatic duct by the aid of secretin was consistently sterile while that collected in a balloon usually contained a few bacteria. These contaminants are not normal inhabitants of the pancreatic duct and may be regarded as opportunists. The fresh juice, that secured by the secretin method, killed the bacteria tested, with the exception of *Staphylococcus aureus*, within forty-eight hours and was more effective than that gathered by the balloon method. In only one instance was digestion of the organisms noted. While *B. tuberculosis* was killed by the pancreatic juice no evidence of dissolution of the cells or loss of acid-fast property was observed. Pancreatic secretion activated with enterokinase was no more bactericidal than the normal juice. Pancreatic juice diluted with salt solution or bile, or made acid, was ineffective against bacteria. The foregoing experiments indicate that the pancreatic juice of the dog has bactericidal properties which may not be great yet may play a part in the normal defense of the pancreas against infection.

AUTHORS' SUMMARY.

ACUTE ANTERIOR POLIOMYELITIS AT VEGA BAJA, PORTO RICO. E. GARRIDO MORALES, J. Infect. Dis. **46**:31, 1930.

Reports of epidemic poliomyelitis in the tropics are rare. Poliomyelitis has probably existed in endemic form in Porto Rico for some time; it has not been recognized or, at least, the cases and deaths have not been reported as such. No epidemic of the disease has ever been reported in Porto Rico before 1928. Poliomyelitis appeared in epidemic form (ten cases, one death) in the town of Vega Baja during April, May and June, 1928. The evidence strongly suggests that indirect personal contact played a major part in the spread of the disease. Apparently, the virus responsible for the present outbreak was not introduced from outside, but from a local source, presumably represented by cases which had occurred two years before in Vega Baja.

AUTHOR'S SUMMARY.

ATTEMPTS TO PRODUCE ACUTE GLOMERULONEPHRITIS IN RABBITS WITH THE PERITONEAL LYSATE OF STREPTOCOCCUS SCARLATINAE. ALLAN F. REITH, LOUIS M. WARFIELD and NORBERT ENZER, J. Infect. Dis. **46**:42, 1930.

In an attempt to repeat the work of Duval and Hibbard on experimental production of acute glomerulonephritis in rabbits we were unable to produce in the peritoneal cavity of immune rabbits a sterile bacteriolysate containing the alleged

endotoxic principle of scarlatinal streptococci. Rabbits given intravenous injection of the filtered or centrifugalized peritoneal fluid of previously immunized rabbits which were given an intraperitoneal mass dose of homologous scarlatinal streptococci from two to three hours before the peritoneal fluid was drawn, did not show symptoms of toxemia. At necropsy the kidneys of some of these rabbits had lesions resembling some of the lesions described by Duval and Hibbard and which they claimed to be the result of acute glomerulonephritis of the scarlet fever type. However, similar lesions were observed by us in the kidneys of normal healthy rabbits and rabbits given injection with bacterial suspension other than scarlatinal streptococci. None of our rabbits showed lesions typical of scarlet fever nephritis.

AUTHORS' SUMMARY.

THE INCIDENCE OF MIDDLE EAR INFECTION AND PNEUMONIA IN ALBINO RATS AT DIFFERENT AGES. JOHN B. NELSON and JOHN W. GOWEN, J. Infect. Dis. **46**:53, 1930.

Data are presented on the incidence of middle ear disease and pneumonia in a colony of albino rats originally established from pneumonia-free breeders and maintained on a balanced diet. The rates of both infections were high in adult rats (1 year and over). In young rats (3 to 4 months) there was a significant but unsymmetrical decrease in the incidence of the two conditions. The respective rats for middle ear infection were 69 and 32 per cent, for pneumonia 81 and 2 per cent. A group of wild rats (approximately 6 months to 1 year) showed 1 per cent middle ear infection and 10 per cent pneumonia. Fifty per cent of the adult rats with middle ear disease had a concurrent inflammation of the nasopharynx while less than 1 per cent of the young rats were similarly affected. The relation between middle ear infection, pneumonia and inflammation of the upper respiratory tract is discussed.

AUTHORS' SUMMARY.

THE BACTERIA OF THE INFECTED MIDDLE EAR IN ADULT AND YOUNG ALBINO RATS. JOHN B. NELSON, J. Infect. Dis. **46**:64, 1930.

The bacterial flora of the middle ear in natural infection in a group of adult albino rats on a balanced diet was found to embrace a wide variety of microorganisms in pure or mixed culture. *B. actinoides*, streptococci and a diphtheroid were most often encountered in the order named. In a group of infected young rats from the same colony there was a greater incidence of sterile cultures. There was likewise a varied bacterial flora which included a diphtheroid, streptococci and *B. actinoides*, in the order of their isolation. Aside from *B. actinoides* and two species of low incidence, all of the bacteria from the middle ear were either observed in direct films or isolated in culture from the nasopharynx of normal young rats. The bacteriologic results are discussed in relation to the etiology of middle ear disease.

AUTHOR'S SUMMARY.

HEAT RESISTANCE OF THE SPORES OF CLOSTRIDIUM BOTULINUM. E. W. SOMMER, J. Infect. Dis. **46**:85, 1930.

Spores of *Cl. botulinum* have been prepared in different mediums under various conditions and heated in phosphate solution of p_H 7 at 100 C. for time intervals of from one-half to five hours. The survivals were determined by subculturing in beef heart medium. Spores grown in 4 per cent peptone showed a resistance of from one and one-half to two and one-half hours. The addition of phosphate raised the heat tolerance to four hours; the effect, however, was not constant. The addition of dextrose and the increase in concentration of peptone raised the number of organisms but not the resistance. After testing numerous substrates a standard casein-digest medium supplemented with Liebig's meat extract was selected as most favorable for the routine production of *Cl. botulinum* spores. A

yield of from 200,000,000 to 500,000,000 spores per cubic centimeter was obtained with a uniformly higher resistance than in any other medium tested. Different lots of casein medium prepared under similar conditions vary in their ability to produce heat resistant organisms. The same lot of medium has consistently yielded identical results. Supplementing with different electrolytes, sand, dextrose, olive oil, protein or vegetable extracts did not improve the basic substrate. Of the fifteen strains tested sixty-two and nineteen type A, gave the most resistant spores. Attempts to increase the resistance by selection failed. Incubation temperatures of 28, 37 and 41 C. showed little influence on the heat resistance of the organism. The most resistant organisms have been found in four to eight day cultures. The degree of anaerobiosis had no evident effect on the heat resistance of the spores. The apparent heat resistance of a spore suspension increases with its density up to approximately 1 billion per cubic centimeter; beyond this limit it may be considered constant. Spores preserved in their own liquor in the icebox and in a dry state usually deteriorate on standing. Numerous products, however, have remained constant over periods of four months. From the distribution curves plotted from forty-six resistance experiments on small spore samples an average heat tolerance of from four to four and one-half hours is shown. . When the results from forty-four pools prepared by the Sharples centrifuge are considered two maxima, at three and one-half and five hours, are evident.

AUTHOR'S SUMMARY.

A CULTURAL STUDY OF CERTAIN ANAEROBIC BUTYRIC-ACID-FORMING BACTERIA. ELIZABETH MCCOY, E. B. FRED, W. H. PETERSON and E. G. HASTINGS, J. Infect. Dis. **46**:118, 1930.

The butyric anaerobes of fermentation form a subgroup of the genus *Clostridium;* they are characterized by their production of acids or of characteristic neutral products in addition. All are granulose-positive, catalase-negative and nonpathogenic. Two general types have been recognized. Group 1 produces acid end-products, chiefly acetic and butyric acids. Of these so-called "true butyric" anaerobes three subtypes have been studied: *Cl. pasteurianum* type, *B. saccharobutyricus* type, and five special *plectridia* (cultures 29 to 33 inclusive). Group 2 produces butyric and acetic acids and the neutral products, butyl and ethyl alcohols and acetone. These are the butyl organisms of industry, *Cl. acetobutylicum* Weizmann. There are certain facultative organisms related to the true anaerobes. In general they produce acetic acid, ethyl alcohol and sometimes acetone, but no butyric acid. *Aerobacillus polymyxa* Prazmowski from Donker is the only representative studied here. The general morphologic and cultural characteristics of butyric, butyl and related facultative organisms have been studied. Some important differences have been noted. Because adequate quantitative studies of the fermentation products have not been attempted, the authors prefer to leave the final classification of the organisms till a future time. At this time three physiologic types of the butyric anaerobes are distinguished: *Cl. pasteurianum* type, *B. saccharobutyricus* type and a *plectridial* type in some respects like the butyl organism, *Cl. acetobutylicum* Weizmann.

AUTHORS' SUMMARY.

RESULTS OF BLOOD CULTURE IN ACUTE POLYARTHRITIS. EDWIN P. JORDAN and JOHN P. BOLAND, J. Infect. Dis. **46**:148, 1930.

Minute bacilli much like those described in this paper have been obtained from the blood and other tissues in many different conditions (Sellards and Bigelow, Mellon, and others). It is also well known that organisms probably wholly unrelated to a disease may be present from time to time in the blood stream. Hence the relationship between organisms found in the blood stream and the disease process in question is only presumptive. Nevertheless, an association which occurs frequently enough in a certain disease, and which does not occur in other diseases, is sufficiently suggestive to warrant consideration. The classifica-

tion of rheumatic conditions is in a state of chaos and it seems possible that further studies may reveal several etiologically distinct groups at present undifferentiated. The unusual frequency with which we have obtained minute bacilli from the blood of patients with acute polyarthritis is worth noting.

AUTHORS' SUMMARY.

RELATION OF PELLICLE FORMATION AND TOXICOGENICITY IN DIPHTHERIA CULTURES. RALPH H. HEEREN, J. Infect. Dis. **46**:161, 1930.

That a definite correlation exists between pellicle and toxicogenicity is shown by the fact that eighty-nine of the 100 toxicogenic colony cultures produced a definite and characteristic pellicle on Wadsworth's medium to which they had become habituated by means of a series of transfers carried over a forty-five day interval. That the pellicle is not necessary for formation of a strong toxin is shown by the fact that the remaining eleven of the 100 toxicogenic colony cultures, carried through the same growth and test conditions, were consistently apellicular. That the increase in toxicogenicity was due to habituation of the cultures to the medium rather than to increased degrees of pellicle formation is shown by the fact that the apellicular forms demonstrated increases similar to those of the pellicular forms. On the other hand it has been shown that pellicle formation in diphtheria cultures does not determine toxicity, since six of fifteen cultures (proved atoxicogenic by two methods) showed pellicles regularly.

AUTHOR'S SUMMARY.

EXPERIMENTAL TRANSMISSION OF ENDEMIC TYPHUS OF THE SOUTHEASTERN ATLANTIC STATES BY THE BODY LOUSE. H. MOOSER and CLYDE DUMMER, J. Infect. Dis. **46**:170, 1930.

The virus of typhus from the southeastern Atlantic States is able to survive and multiply in the body louse. The louse, therefore, must be considered as a possible factor in the epidemiology of typhus in southern United States.

AUTHORS' SUMMARY.

FILTRABILITY OF VIRUS OF PSITTACOSIS IN BIRDS. CHARLES ARMSTRONG, G. W. MCCOY and SARA E. BRANHAM, Pub. Health Rep. **45**:725, 1930.

Experiments are reported the results of which indicate that the causative agent of psittacosis in birds is filtrable.

RICKETTSIA-LIKE INCLUSIONS IN PSITTACOSIS LESIONS. R. D. LILLIE, Pub. Health Rep. **45**:773, 1930.

The lesions seen in three parrots associated directly or indirectly with human cases of psittacosis are described and briefly compared with those in a human case. Minute intracellular inclusions are described in human lesions and in the lesions in the parrots, and the name *Rickettsia psittaci* is proposed for them. The evidence of a laboratory outbreak of the disease indicates that the virus (sensu lato) of psittacosis was present in some of the birds under investigation.

AUTHOR'S SUMMARY.

ACCIDENTAL PSITTACOSIS INFECTION AMONG THE PERSONNEL OF THE HYGIENIC LABORATORY. G. W. MCCOY, Pub. Health Rep. **45**:843, 1930.

Of eleven cases (between January 25 and March 15, 1930) two occurred in persons handling infected birds, one in a person who worked only with cultures from infected birds, but none of the eight remaining cases could be traced to any recognizable source of infection. All of these persons, however, worked in the building in which the work on psittacosis was carried on in certain rooms to

which only those engaged in the work had access. The usual precautions employed in studies of dangerous infections were carried out. These examples of infection through the medium of contaminated environment without contact with infected birds suggest "that the infectiveness of the virus of psittacosis for man is of a very high order."

A New Meningococcus-Like Organism from Epidemic Meningitis. Sara E. Branham, Pub. Health Rep. **45**:845, 1930.

During an epidemic of cerebrospinal meningitis in which all four of the usual types of meningococci were involved, an apparently new form was isolated from the spinal fluid of fourteen cases. In morphology this micro-organism is indistinguishable from the other members of the genus *Neisseria*. It differs from the meningococcus in pigment production, lack of fermentative action and antigenic relationship. These fourteen strains form a homogeneous group culturally, biochemically and serologically. The name *Neisseria flavescens* n. sp. is proposed for this new form. Since 30 per cent of the spinal fluid strains received from this locality belong to this group (comprising 9 per cent of the total number of strains received at the Hygienic Laboratory during 1928-1929), since it is not represented in any of the therapeutic polyvalent serums now manufactured and since the mortality in these cases was at least 30 per cent, the occurrence of *N. flavescens* in epidemic meningitis warrants special attention.

A Bacteriological and Experimental Study of Cholecystitis. A. C. Nickel and E. S. Judd, Surg. Gynec. Obst. **50**:655, 1930.

Green-producing streptococci, gram-negative bacilli and staphylococci were isolated from the majority of acutely or subacutely inflamed surgically removed gallbladders. Cultures from the "strawberry" and chronically infected bladders were found to be sterile in most instances, unless there was a complicating factor. The streptococci obtained were capable of producing the disease in rabbits when injected intravenously.

Richard A. Lifvendahl.

Treatment of Experimental Tuberculosis by Calcium Administration. J. C. Hoyle, Quart. J. Med. **22**:451, 1929.

The experiments give no support to the view that the oral administration of calcium has any beneficial effect on tuberculosis. Likewise, the intravenous administration of calcium had no beneficial effect. Rabbits were inoculated with a virulent bovine strain and were then given intravenous injections of calcium chloride. As many as forty-eight injections were given. The average length of survival after inoculation in control rabbits was fifty days; in treated ones, forty-six days. At postmortem examination no differences were noted in either the extent or the character of the disease in the two series.

N. Enzer.

After-Results of Gassing and Gunshot Wounds of the Chest in Relation to Tuberculosis. G. Basil Price, Tubercle **11**:97, 1929.

While "gassing" appears to bear a relatively unimportant part on the incidence of tubercle, in gunshot wound injuries of the chest (all types, surface and penetrating) the incidence of pulmonary tuberculosis forms a small but appreciable percentage of not less than 0.5. This is more than double that occurring among the civil population. If the relative incidence of pulmonary tuberculosis could be applied to men suffering from only penetrating injuries of the chest, this percentage of 0.5 would in all probability be considerably exceeded. When the pulmonary tissue has been definitely injured, and especially when a foreign body is retained in or near the damaged area, activation of tubercle may occur in the organ affected, even at an indefinite period later.

H. J. Corper.

IS THE VIRUS OF TRACHOMA FILTRABLE? M. C. TRAPESONTZEWA, Ann. d'ocul. **167**:160, 1930.

Trapesontzewa obtained trachomatous material in large quantities and filtered it with the least possible addition of salt solution. Blind human subjects were used for a part of the experiments. From material procured by scraping, excision and expression and by trituration with but a few drops of physiologic solution of sodium chloride, she obtained a filtrate, using tiny filters specially made in Germany. Inoculations were made by subconjunctival injections or instillations into the scarified conjunctiva. Not a trace of a follicle was found. In one experiment the author herself was the subject. The material was taken from twelve florid cases and inoculated by two ophthalmologists. Within about thirty-six hours the edema of the upper lid, as well as the conjunctival injection, disappeared. Two weeks later the other eye was inoculated, after scarification of the conjunctiva, and a portion of the filtrate was inoculated, subconjunctivally, into the first eye. Within a few days the mild inflammation was gone. A month later a filtrate was made from the material obtained from twenty-four trachomatous lids that had never been treated. Subconjunctival injection and instillation into the scarified conjunctiva were made, and the conjunctiva was examined every day. The last examination, made six months later, revealed neither granulations nor follicles. The author concludes that the virus of trachoma is nonfiltrable.

CHARLES WEISS.

CONDITIONS REQUISITE FOR THE PRODUCTION OF LOCAL IMMUNIZATION IN INFECTIONS OF THE EYE. L. POLEFF, Arch. f. Augenh. **102**:722, 1930.

It has been demonstrated that certain tissues of the eye, especially the bulbar conjunctiva, the cornea and the anterior chamber, can produce local specific antibodies, and this function is of special significance in those infections that stand out because of their strong ophthalmotropism, e. g., the virus of trachoma, diplobacillus infections and possibly gonorrheal ophthalmia. The other organisms (staphylococcus, gonococcus and streptococcus) are known to induce only a local immunity, even in other organs of the body, which is not accompanied by antibodies in the blood. Poleff discusses the use of Besredka's bouillon filtrates in producing local immunity and points out the basis for such investigations:

Broth culture filtrates have a species-specific, growth-arresting character for homologous organisms in vitro.

In experimental animals they produce a local, sharply limited and specific immunity which protects them against repeated lethal doses of the organism.

This immunity, produced only by the use of specialized technic, occurs when the filtrate comes into direct and sufficiently prolonged contact with the tissue that is to be immunized. Finally, a virulent strain pathogenic for the eye should be selected. (For the present, staphylococcus or streptococcus filtrates only may be used in the eye.)

The best results are obtained by injecting the "antivirus" subconjunctivally, or by applying it as a salve. After repeated injections the immunity advances to the limbus and protects it against phlyctenulae which are so easily formed in tuberculous animals that are subjected to staphylococci. Immunizing the cornea is more difficult because of its retarded metabolism. Only after repeated subconjunctival injections of 1 per cent sodium chloride solution was the instillation of the filtrate followed by a positive result. The author recommends the addition of atropine to the antivirus in order to prevent absorption of the immunizing factor. The vitreous is immunized only by deep peribulbar and retrobulbar injections of the filtrate.

In all these cases the immunity obtained is neither absolute nor permanent. Antivirus has been successfully used in many hundreds of cases of ulcerative blepharitis, dacryocystitis, corneal ulcer, recurrent hordeolum, postoperative infection, etc.

CHARLES WEISS.

THE TUBERCULOTOXIC NATURE OF PHLYCTENA AND OTHER SCROFULOUS MANIFESTATIONS. H. GUILLERY, Virchows Arch. f. path. Anat. **273**:806, 1929.

Typical phlyctenae can be produced in the rabbit's eye without working on the eye itself, if one tuberculinizes the rabbit and injects bacterial ferments which make hyperemia of the eye. At autopsy the animals were found free from tuberculous lesions; the phlyctenae contained no tubercle bacilli and no necroses. A small, permeable bag was inserted behind the one eye, and a phlyctena resulted in the other eye; abdominal insertion also led to phlyctena. Microscopically, one finds many small phlyctenae, besides the few large ones, and much perivascular infiltration in the bulbar conjunctiva. Similar cell masses were found in sclera, ocular muscles and orbital fat. Corresponding examinations have not been made on eyes of scrofulous children. In some rabbits, corneal phlyctenae were found. In all animals into which a bag of tubercle bacilli was inserted, the tuberculin reaction became positive. Inflammatory processes in the surroundings of the bag indicate the way the tuberculotoxins are taking.

ALFRED PLAUT.

MUCOR MYCOSIS IN SWINE. M. CHRISTIANSEN, Virchows Arch. f. path. Anat. **273**:829, 1929.

Mold diseases in domestic animals are even more rare than in man. In nine hogs that were about 6 months old, tumor-like masses and abscesses were present in the abdomen, mostly coming from the mesenteric lymph nodes. There were smaller nodes in the liver and the lungs; some animals had intestinal ulcers with thick margins; occasionally, nodules in other organs and extra-abdominal, mostly cervical, lymph nodes were found. The intestinal lesions sprang from the plaques of Peyer. Seven of the nine animals came from one slaughter house. Single animals had the disease, while others from the same litter were free. The hyphens of the fungi were easily found in fresh specimens. Pure cultures were obtained from all the animals. The cultures were highly pathogenic for rabbits, guinea-pigs, rats and mice, but were not pathogenic for sparrows and pigeons. Rabbits, after intravenous injection, developed labyrinthine symptoms as described in *Aspergillus* infection. Inoculation of young pigs and of a pregnant sow did not lead to development of the disease. Some of the young animals died after intravenous injection, and subcutaneous injection occasionally led to a localized abscess.

ALFRED PLAUT.

EXPERIMENTAL INVESTIGATIONS OF POSTVACCINAL ENCEPHALITIS. J. P. BIJL AND H. S. FRENKEL, Zentralbl. f. Bakteriol. (Abt. 1) **112**:412, 1929.

The authors injected the neurovaccine of Gallardo into rabbits cutaneously and studied the effects. A large proportion of the animals died about the tenth day after inoculation. In these rabbits miliary foci of inflammation were found in various organs of ectodermal, mesodermal and entodermal origin. In the same organs the vaccine virus was recovered. The foci, especially in the lungs, are characterized by a marked proliferative reaction with a perivascular infiltration of mesenchymal cells and, in the opinion of the authors, are specific for the neurovaccine of Gallardo. The suggestion is made that the similar foci of inflammation in the brain in cases of encephalitis are caused by the vaccine virus or its toxins.

PAUL R. CANNON.

THE ETIOLOGY OF GRIP. D. A. PAVLOVIĆ, Zentralbl. f. Bakteriol. (Abt. 1) **112**:429, 1929.

Secretions from the throats of patients with grip were injected intratracheally into rabbits and produced fever, inflammation and punctate hemorrhages in the lungs. The results were the same with both unfiltered secretions and with those

passed through a Chamberland filter L_2. The secretions were usually taken during the first twenty-four hours of the illness. The pathologic observations were similar to those observed by Olitsky and Gates, viz., hyperemia of the tracheal and laryngeal mucosa with punctate hemorrhages in the pleural surfaces and deep in the lungs. Passage of the virus from animal to animal was unsuccessful. Animals once infected, however, were immune to a second injection of the virus. Using the technic employed by Olitsky and Gates, unsuccessful attempts were made to isolate *Bacterium pneumosintes*. Another organism, however, was isolated which is called by the author *Bacterium granuliformans*. This organism is a facultative anaerobe and is named *granuliformans* because of the presence of small granules after from ten to fifteen days of cultivation in broth. While Pavlović does not claim that this bacterium is the cause of grip, he believes that it and *B. pneumosintes*, which it resembles, are related to grip in some manner.

PAUL R. CANNON.

THE NATURE OF ANTIVIRUS. B. G. MATWEJEWSKY, Zentralbl. f. Bakteriol. (Abt. 1) **112**:464, 1929.

The author studied the problem of the nature of the growth inhibition in vitro of Besredka's so-called antivirus, using staphylococcic and streptococcic filtrates. The addition of sources of energy such as carbohydrate or broth to the filtrate led to growth of the organisms, whereas the addition of peptone or ascitic fluid did not. Adjustments of the p_H did not favor growth of the bacteria. The growth-inhibiting effect was nonspecific and seemed to be due to the exhaustion of the mediums, especially of sources of energy such as are present in broth and carbohydrates.

PAUL R. CANNON.

ATTEMPTS AT INFECTION BY RUBBING INFECTIOUS AGENTS INTO THE INTACT AND THE SUPERFICIALLY TRAUMATIZED SKIN. Y. S. SHOUKRI, Ztschr. f. Hyg. u. Infektionskr. **110**:697, 1929.

Infection by way of the scarified skin occurs less readily than parenteral infection by way of the subcutaneous tissue or the peritoneum, but much more easily than infection by way of the intact skin. A complete severance of the skin does not increase the chances of infection but seems to lessen them to some extent. Infection from the scarified skin was rarely due to a few single organisms. As a rule, it required from 100 to 1,000 times more than the smallest infectious dose in subcutaneous or intraperitoneal infection. Smaller quantities were either ineffective or resulted in a more protracted course which seemed to favor the development of metastatic foci in the pericardium, the pleura or the lungs.

W. OPHÜLS.

THE SCARLATINOTOXIC PROPERTIES OF HEMOLYTIC STREPTOCOCCI FROM CASES OF ANGINA. W. GRUNKE and E. BARTH, Ztschr. f. Hyg. u. Infektionskr. **110**:738, 1929.

Filtrates were made of cultures of hemolytic streptococci obtained from the throats of twenty-one patients with angina lacunaris or angina phlegmonosa. These were compared with standard Dick toxin by skin reaction in thirty-seven persons. In 50 per cent of the cases the skin reaction was identical.

W. OPHÜLS.

ENCEPHALITIS IN RABBITS WITH COCCIDIOSIS. B. GALLI-VALERIO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **65**:325, 1930.

Meningo-encephalomyelitis may develop in rabbits with intestinal coccidiosis. The lesions in the central nervous system are due probably to toxic products of *Coccidia*.

Immunology

BIOLOGICAL TESTS FOR HYDATID DISEASE. R. H. GOODALE AND H. KRISCHNER, Am. J. Trop. Med. **10**:71, 1930.

In a series of 106 cows, both the intradermal and complement fixation tests for hydatid disease were performed to compare their relative values. These tests were checked by examination of the organs after slaughtering. Of forty-four cows in which hydatid cysts were found, thirty-eight, or 86.3 per cent, gave a positive skin test, and twenty-six, or 59 per cent, a positive complement-fixation test. There were eleven false positive skin tests and ten false positive complement-fixation tests. In the rest of the series the observations substantiated those in the organs. One of the two tests was positive in all of the cows in which hydatid cysts were found.

AUTHORS' SUMMARY.

ON THE HEREDITY OF THE LANDSTEINER BLOOD GROUPS. ALEXANDER S. WIENER, MAX LEDERER and S. H. POLAYES, J. Immunol. **18**:201, 1930.

The bloods of 1,334 mothers and their 1,462 children were typed. Although 485 group 0 mothers with 516 children and 94 group AB mothers with 142 children were examined, not once did the combinations AB mother—0 child or 0 mother—AB child appear. The authors' data, together with Schiff's and Thomsen's, therefore completely support the Bernstein theory. A survey of the literature shows that many apparent exceptions were found. On careful analysis of these cases, not a single completely proved exception to Bernstein's theory could be found. It may therefore be concluded that although the existence of bona fide exceptions to the Bernstein theory cannot be disproved entirely, it is certainly true that of all the theories of heredity of blood groups, Bernstein's theory agrees best with the facts. Any true exceptions to this theory may be due to other factors complicating the mechanism of heredity.

AUTHORS' SUMMARY.

SPECIFICNESS OF SENSITIVENESS (TUBERCULIN TYPE) TO EGG PROTEINS. L. DIENES, J. Immunol. **18**:279, 1930.

According to the observations described in this paper, the sensitiveness of the tuberculin type produced in guinea-pigs with egg globulin, crystalline egg albumin, and ovomucoid is specific to the preparation with which the animals were treated. The specificity of this type of sensitiveness, like that of the anaphylactic sensitiveness, corresponds to the antigen specificity.

AUTHOR'S SUMMARY.

ANTIGENS IN SYNTHETIC MEDIUM FOR TUBERCLE BACILLUS. L. DIENES and E. W. SCHOENHEIT, J. Immunol. **18**:285, 1930.

In the filtrates of cultures grown on the synthetic medium of Long, beside the carbohydrate precipitable substance we can demonstrate the presence of two antigenic substances. They are separated from each other by acid precipitation. By both the serologic reactions and the antibody production these antigens are well differentiated from each other and from the other antigens of the tubercle bacillus. The difference between these two fractions can be observed also concerning the tuberculin reaction in guinea-pigs treated with one or the other of these preparations. The acid-precipitable antigen readily forms antibodies in tuberculous animals which are specific toward this antigen. The acid nonprecipitable fractions did not form antibodies reacting with the same preparations or with the acid precipitate and concentrated culture medium, but the serums of the majority of the guinea-pigs treated with these preparations gave strong reactions with the bacillary emulsion. From the observations that neither of the two antigens separated by acid precipitation or their mixture gave a positive reaction with several serums produced with the concentrated culture medium and that the latter did not react

with the serums produced with the acid precipitate we draw the conclusion that in the culture medium the antigens that are separated by acid precipitation are united in a higher complex which has a specifically markedly different from that of the isolated antigens, and in the reactions of which the effect of the isolated antigens does not appear. The differences that we found concerning the serologic reactions between the culture filtrate of different tubercle bacillus strains are probably caused not by the absence or presence of a certain antigen, but by the differences in the way in which the acid-precipitable part is united to the higher complex. Probably the aforementioned complex antigen is responsible for the production of antibodies by the concentrated culture medium and the acid filtrate, although this complex often gives no reaction or only a slight one with these serums.

AUTHORS' SUMMARY.

INFLUENCE OF AGE UPON ANTIBODY FORMATION. JULES FREUND, J. Immunol. **18**:315, 1930.

In rabbits less than 20 days of age, the formation of agglutinins against typhoid bacilli, of hemolysins against sheep cells, and of precipitins against horse serum and egg white is strikingly less intense than in adult rabbits immunized in the same way. A definite Arthus' phenomenon cannot be produced in young rabbits immunized with horse serum or egg white.

AUTHOR'S SUMMARY.

THE ANTIBODY CONTENT OF THE BILE OF IMMUNIZED RABBITS. JULES FREUND and HOWARD J. HENDERSON, J. Immunol. **18**:325, 1930.

Antibodies are eliminated from the liver in the bile of rabbits actively or passively immunized against typhoid bacilli. The following numerical relation exists between the agglutinin titers of the serum, liver extract, lymph of the liver and bile, expressed in percentages: serum 100 per cent, liver extract 10 per cent, lymph of liver 80 per cent and bile 0.8 pr cent. In the bile of passively immunized rabbits the maximum titer is reached within two hours after the immune serum has been injected. The loss of antibodies by elimination with the bile explains at least in part why antibodies disappear from the blood of animals after active or passive immunization.

AUTHORS' SUMMARY.

PREPARATION AND ANTIGENIC PROPERTIES OF CARBONMONOXIDE HEMOGLOBIN. ALDEN KINNEY BOOR and LUDVIG HEKTOEN, J. Infect. Dis. **46**:1, 1930.

Pure carbonmonoxide hemoglobin was prepared by treating oxyhemoglobin, made by the method of Marshall and Welker, with pure carbonmonoxide, and in all species used, except man, the carbonmonoxide hemoglobin was recrystallized from alcohol three or four times at O C. The watery solutions or dried crystals were preserved at O C. and at room temperature under an atmosphere of carbonmonoxide. The report of previous investigators that oxyhemoglobin is antigenic and mainly species-specific was confirmed by the precipitin test, and likewise carbonmonoxide hemoglobin was found to be antigenic and mainly species-specific. Cross reactions appeared in the case of closely related species, such as duck, chicken and turkey, beef and sheep. Cross reactions of one dog antiserum and one sheep antiserum with other species were observed. These antiserums became species-specific when diluted 1:3, which suggests a common radical in the antigen having weaker antigenic powers than the species-specific portion of the molecule. This point requires further work.

AUTHORS' SUMMARY.

ON THE PURIFICATION AND CONCENTRATION OF SCARLET FEVER TOXIN. LAWRANCE E. SHINN, J. Infect. Dis. **46**:76, 1930.

The experiments reported here indicate that a lethal dose of scarlet fever toxin for rabbits has not been established. Although rabbits were given intravenous

injections with more than seven times the dosage of scarlet fever toxin designated by Hartley as the lethal dose for rabbits, no specific lethal action of the toxin was demonstrated. It was possible to concentrate scarlet fever toxin by precipitation with sodium sulphate and acetic acid. Attempts to obtain further purification by fractional precipitation with sodium sulphate were unsuccessful. Attempts to concentrate scarlet fever toxin by precipitation with acetic acid alone were not successful. Precipitation with acidified alcohol gave the most uniform and the best results. Rabbits are not suitable for standardization of scarlet fever products. Concentration of scarlet fever toxin may be obtained by several methods. Of those used in these experiments, precipitation with alcohol acidified with acetic acid gave the best results.

AUTHOR'S SUMMARY.

THE COMPOSITION OF CRYSTALLINE PROTEINS FROM HUMAN BLOOD SERUM AND URINE. HILDA H. KROEGER and LUDVIG HEKTOEN, J. Infect. Dis. **46**:115, 1930.

On successive crystallization in filtrates of ammonium sulphate precipitations of proteins in human blood serum, albumin tends to become the chief constituent of the globular crystals as determined by their precipitin reactions. Crystallization consequently may be a means of separating albumin from the globins in blood serum. In nephritic proteinuria a large part of the protein proved to be albumin.

AUTHORS' SUMMARY.

THE ZONE PHENOMENON IN AGGLUTINATION TESTS. R. R. SPENCER, J. Infect. Dis. **46**:138, 1930.

Studies were made of a serum which agglutinated *Brucella abortus* in high dilutions and showed an unusual middle zone of inhibition. The middle zone of inhibition could be transferred to other positive *abortus* serums but not to anti-typhoid serums, suggesting that the zone phenomenon is a specific reaction. However, a later specimen of serum from the same patient gave a wide prezone which could not be transferred to other anti-*abortus* serums. The presence or absence of the zone was controlled by the temperature at which the agglutination test was incubated. This was found to be true also for an antimeningococcus serum exhibiting a zone. The optiumum temperature for obtaining the zone, however, was not the same for both serums. Inactivation at 56 C. of the serum showing the zone tends to increase its range. A prezone may be induced in some positive serums, but not in all, by inactivating them at 56 C. These tests serve to emphasize the great difficulty in making trustworthy generalizations covering even a single antigen-antibody reaction (agglutination) since the knowledge concerning the nature of such immune reactions is still so incomplete. Therefore it is preferred for the present to report the results without further comment.

AUTHOR'S SUMMARY.

ON ISOHEMAGGLUTINATION, THE HEMOLYTIC INDEX AND HETEROHEMAGGLUTINATION. LELAND W. PARR, J. Infect. Dis. **46**:173, 1930.

Data for 1,685 serologically syphilitic serums tested for blood groups fail to support the contention that there is any relation between any one blood group and susceptibility to or resistance against syphilic infection. Furthermore, the small amount of dementia paralytica in the Near East where 10 per cent of the people are of the blood group AB speaks against any marked increase of late syphilis in persons belonging to that blood group. Similarly, negative observations are reported as regards the relationship of malarial infection to any one of the four blood groups, based on a study of 279 cases of malaria. While my data on the blood groups and sex, based on a series of 7,074 cases, indicate a slight preponderance of females in blood group AB, yet there is probably no significance to this observation in view of the contradictory nature of other observations on the subject and the absence of any theoretical reason for such an increase. Racial

blood group data for the French of the poorer classes of Paris show the unsatisfactory nature of such data for racial studies in view of the wide variations encountered by different workers dealing with the same group of pople. Fresh blood serum of more than 93 per cent of Near Easterners has hemolytic activity against sheep red blood cells. The average hemolytic index is 4.12, based on 788 determinations. There is no significant racial difference, but blood group 0 persons average greater hemolytic power than others whereas AB people have least of this power. The blood of new-born infants has no hemolytic activity; this is acquired after birth, whereas the content of complement and isohemoagglutinin is partially present at birth. Attention is called to the high percentage of pregnant women who at term give a hemolytic index of zero. The almost universal occurrence of heterohemagglutination between certain animal serums and heterologous red blood cells was confirmed. Positive reactions occurred in 99.6 per cent of 1,485 tests made on cattle, pig and rabbit serums in contact with rabbit cells and especially with human cells of all four groups.

AUTHOR'S SUMMARY.

BRUCELLA AGGLUTININS IN THE BLOOD AND MILK OF COWS. ROBERT GRAHAM and FRANK THORP, J. Infect. Dis. **46**:260, 1930.

It is apparent that a negative agglutination test with milk serum is not a reliable indication of the *Brucella* agglutinin content of the blood of the same animal. Agglutination with the milk serum detected from 47 to 68 per cent of the cows in two *Brucella*-infected herds, the blood serum of which reacted. These observations tend to confirm the generally accepted limitations of the milk serum test for *Brucella* agglutinins in the diagnosis of this infection in cattle.

AUTHORS' SUMMARY.

NEW BLOOD GEN (A') AND RESULTING BLOOD GROUPS A' AND A'B. O. THOMSEN, V. FRIEDENREICH and E. WORSAAE, Klin. Wchnschr. **9**:67, 1930.

The authors believe that another blood gene A' has been demonstrated, and that instead of only four groups there are six, namely, O, A, A', B, AB, and A'B. The two new groups are not subgroups, but rather, independent and as important as the other four. A technic for the demonstration of these two new groups is described.

AUTHORS' SUMMARY.

Tumors

ON THE CHANGES IN THE MAMMARY GLAND PRECEDING CARCINOMA. ALEXANDER A. CHARTERIS, J. Path. & Bact. **33**:101, 1930.

A histologic study of forty-eight breasts removed by operation for carcinoma has been carried out. Of these, forty-one were in addition the seat of so-called chronic cystic mastitis, and this lesion is described in detail; except in two cases the diagnosis was possible only on slicing up the organ or on microscopic examination. All grades of epithelial hyperplasia may be found in the ducts and acini of the organ, and the earlier stages are usually to be seen in association with the "chronic mastitis." The process may result in the formation of papillomatous growths wth a variable amount of stroma or in more cellular and diffuse growth without stroma. The more purely cellular hyperplasias may be traced through a series of progressive developments in which changes in the character of the cells are at last apparent. No line of demarcation between the various stages can be made out; they merge insensibly with each other until at last the ducts and acini are filled with cells indistinguishable histologically from cancer cells—intraduct carcinoma. These finally break through the duct wall and invade the tissues, forming a cancerous tumor. This is the common sequence of events. Cancer, however, occasionally arises in connection with papillomas, and those of the filiform variety with a minimal amount of stroma seem especially prone to undergo malignant transformation, giving rise to the cancer in two cases of this series. The

malignant growth is believed to have arisen from the ducts in thirty-one of the forty-eight cases. Paget's disease of the nipple is referred to, and one case in particular provides some interesting facts bearing on the relationship between this lesion and intraduct carcinoma. The occurrence of desquamative changes and of epithelium of sweat gland type is briefly discussed. From these observations it would appear that the onset of cancer in the breast is frequently the result of a long series of proliferative changes, mainly in the duct epithelium, and that these begin as relatively simple lesions the study of which might give information of value as regards prophylaxis. More work of an experimental nature is necessary.

AUTHOR'S SUMMARY.

FURTHER EXPERIMENTS ON THE CARCINOGENICITY OF SYNTHETIC TARS AND THEIR FRACTIONS. C. C. TWORT and J. D. FULTON, J. Path. & Bact. **33**: 119, 1930.

The carcinogenic activity of synthetic tars varies according to the chemical compound utilized for their manufacture. A synthetic tar made from pinene at 850 C. was a more powerful carcinogenic agent than one made from turpentine under similar conditions. The relative carcinogenic potencies were 100 and 21, respectively. The carcinogenic activity of a synthetic tar varies according to the temperature of the combustion tube. Pinene tars made at 500, 600, 750, 850 and 950 C. had relative potencies of 0, 12, 24, 100 and 64. A synthetic tar was concentrated as far as its carcinogenic activity was concerned by means of distillation and differential solubility in alcohol. A 5 per cent solution of our most concentrated fraction was about five times as powerful as the ordinary crude gas tars tested by us. The active constituents in the synthetic tar formed crystalline picrates; the extract proved to be almost devoid of carcinogenic activity. The trinitrophenol extract proved to be extremely potent as a cancer-producing agent. Oxygenation of a synthetic tar at 100 C. reduced the potency of the tar from 100 to 63, and when the same tar was oxygenated at from 150 to 160 C. the potency fell to 1. Oxidation with an acetone solution of permanganate reduced the potency to 24; with pyridine as a solvent the figure fell in one case almost to 0. Reduction of the tar by means of sodium in boiling amyl alcohol reduced the potency of the tar to 0.5, and when this tar was treated with sulphur its potency was raised to 5. Dilution of the tar with oleic acid instead of with the inert liquid paraffin reduced the potency by about 20 per cent. Similarly, oleic acid reduced the potency of coal gas tar from 90 to 66. Applications of oleic acid have given rise to benign tumors. With lactic or butyric acid, no tumors were observed. A mixture of hydrocarbons soluble in chloroform failed to induce the development of tumors, while a mixture of insoluble hydrocarbons, suspended in liquid paraffin, gave rise to two epitheliomas. Among several insoluble hydrocarbons tested in the pure state, suspended in liquid paraffin or oleic acid, chrysene (if our specimens have been completely purified) appears definitely to be carcinogenic although its potency is extremely low. Among liquid hydrocarbons tested American turpentine and pinene have both induced the development of a benign tumor.

AUTHORS' SUMMARY.

TAR LESIONS BY THE INTRAVENOUS ROUTE. CESARE TEDESCHI, Tumori **4**: 101, 1930.

The author has subjected rabbits to repeated intravenous injections of tar in oil and has made a histologic examination of their viscera. He emphasizes especially regressive processes in the heart, the lungs and the liver and the frequent formation of sclerotic foci; he has encountered more rarely the signs of an inflammatory process. In fibrous spots of the pulmonary parenchyma hyperplastic changes of the alveolar epithelium are observed; hyperplasia with a somewhat atypical character is also seen in the epithelium of the small bronchi. The observations show a general toxic action of tar.

AUTHOR'S SUMMARY.

STUDY ON THE BASAL METABOLISM IN PATIENTS WITH TUMOR. DINO DONATI, Tumori **4**:126, 1930.

In studying the basal metabolism in cases of tumor, a diminution was found in patients with cancer, sensibly diminished values in two persons with benign tumors and an increase in cases of sarcoma and lymphosarcoma. An increase was also found in a case of myelocytic leukemia. Radium therapy produced an increase of from 15 to 20 per cent, roentgen therapy, an increase of from 6 to 10 per cent. In both cases the increase was noted only on the day following the application.

AUTHOR'S SUMMARY.

GANGLIONEUROMA OF THE PINEAL BODY. A. SCHMINCKE, Beitr. z. path. Anat. u. z. allg. Path. **83**:279, 1929.

One-half year before his death, a man, aged 50, began to complain of headache, dizziness, diplopia and sleepiness. Three months before death, his gait became unsteady. A decompression operation resulted in death. On his admission to the hospital, there was slight paresis of the left facial and abducens nerves, spontaneous nystagmus to the right, absence of the cremasteric reflex, increased patellar reflexes and a positive left Babinski sign. Ventriculography revealed compression of the posterior horn of the right lateral cerebral ventricle. At necropsy, the pineal body was the size of a small cherry. Attached to and a part of the pineal body was a rounded tumor mass, which rested on the corpora quadrigemina and which had caused obstruction of the ventricular system. Histologic examination showed the tumor to be composed of glia cells, many of them of giant size, glia fibers, ganglion cells in various stages of differentiation, and nonmedullated axis cylinders. The only change noted in the genital system was a moderate increase in the size of the testes, in which spermatogenesis was active.

O. T. SCHULTZ.

PRECANCEROUS CHANGES IN BLOOD VESSELS. LEIV KREYBERG, Virchows Arch. f. path. Anat. **273**:367, 1929.

After reviewing some pertinent questions of the present knowledge of experimental carcinoma, Kreyberg formulates his problems as follows: What are the local anatomic and functional changes in the cutaneous blood vessels of the white mouse after painting with tar? What rôle may these changes play in epithelial proliferation and cancer formation? Nonpregnant white mice were used. The tar used had been found potent in previous experiments. The vessels were studied in the living mouse, partly under a binocular microscope. A 1:3 per cent aqueous solution of sodium sulphide keeps the skin hair free and makes it translucent. The mild hyperemia caused by the sulphide does not interfere with the vascular changes. Preparations of skin were examined in balsam; they mostly were taken from mice into which carmine had been injected. There was always transudation in the tar-painted skin area; the small vessels were much distended. The hyperemia began immediately after the first painting. It lasted for about forty-eight hours. After repeated paintings the hypermia lasts longer, its duration corresponding to the duration of painting. It occurred also in areas that were cut off from their nerve supply. The tar obviously damages the contractile apparatus of small vessels. When the tar painting is not continued for more than one month, the blood vessels recover. Otherwise permanent dilatation is established, accompanied by transudation. Painting with spiritus sinapis did not lead to permanent vascular changes; neither did regular application of hot water. The local action of the tar was characterized by intense hyperemia and relatively slight toxic action. In rabbits and rats there was no vascular reaction after painting with tar, and no hyperplasia of epidermis. The hyperemia appears earlier than the small warts. It may, together with other factors, lead to local increase of growth. In addition, there is the local and general toxic action of the tar. The possibility of a connection between tar hyperemia and tar hyperplasia is stressed.

ALFRED PLAUT.

A Rare Tumor of the Cecum in an Inoculated Mouse. L. Heidenhain, Virchows Arch. f. path. Anat. **273**:541, 1929.

Ten mice were treated intramuscularly with fresh suspension of a congenital myelogenous giant cell sarcoma. In six of the ten mice malignant tumors developed. One of the mice had the following tumors: squamous cell carcinoma of the colon; carcinoma of the ileum, uterus and pylorus; double carcinoma in the cecum, and sarcomatous lymph nodes in the region of the pancreas.

Alfred Plaut.

Tobacco and Tobacco Smoke as Etiologic Factors in Cancer. F. Lickint, Zschr. f. Krebsforsch. **30**:349, 1929.

The author gives a careful and excellent survey of the literature on the relationship of tobacco to cancer. He summarizes the knowledge of the subject as follows: Experimentally, cancer has not as yet been induced by the application of tobacco tar, although this has been found to cause epithelial hyperplasia. From clinical observation, tobacco may be regarded as a cause of cancer of the lip, tongue, oral mucosa, gums, pharynx, esophagus and stomach, as well as of the larynx, bronchi and lungs in the respiratory tract. He inclines to the view that it may occasionally be responsible for primary cancer of the liver and urinary bladder.

H. E. Eggers.

Medicolegal Pathology

Purified Mineral Oils Not Carcinogenic. F. C. Wood, J. A. M. A. **94**:1641, 1930.

Extensive appropriate experiments on white mice and rats with purified mineral oils (Squibb's Liquid Petrolatum and with Nujol) failed to produce carcinoma of the gastro-intestinal tract. These negative results favor the assumption that purified mineral oils do not produce carcinoma in man.

Methyl Salicylate Poisoning in Infancy. Irwin S. Meyerhoff, J. A. M. A. **94**:1751, 1930.

A boy, aged 22 months, swallowed about 24 cc. of synthetic methyl salicylate. Vomiting was an early symptom; the breath smelled of wintergreen; the abdomen was distended. After death, the lungs were congested; the liver large, smooth, pale and yellow; the lining of the stomach was edematous, and the contents of the ileum and cecum had the odor of methyl salicylate. Microscopically, early glomerular nephritis, degeneration of the liver, and hyperplasia of lymphatic structures in the ileum and of mesenteric lymph nodes were noted.

Review of Carbon Monoxide Poisoning. By R. R. Sayers, Surgeon United States Public Health Service, and Sara J. Davenport, Principal Translator, United States Bureau of Mines. Prepared by direction of the Surgeon General. United States Treasury Department, Public Health Service. Public Health Bulletin, no. 195. Paper. Price, 20 cents. Pp. 97. Washington, D. C.: Superintendent of Documents, Government Printing Office, 1930.

The various aspects of carbon monoxide are considered — occurrence, symptoms, diagnosis, pathology, prevention and treatment. The bibliography at the end contains 195 references, which are not arranged alphabetically. There are many interesting reminders of the historical side of this ancient form of poisoning. The medicolegal problems of monoxide poisoning are not discussed systematically. Under the heading of pathology are considered mainly the various opinions in

regard to the genesis of the symptoms of the poisoning and of the lesions in the brain, which are now regarded as the result of anoxemia and secondary vascular changes. Pathologic anatomy is not described minutely and systematically. The percentage of carbon monoxide that is dangerous to breathe is considered fully, as are also the methods for determining its presence in the blood and in the air. The medicolegal pathologist will be interested in the method for determining carbon monoxide in the blood, devised by Sayers and his collaborators. This method is based on the fact "that a light gray brown suspension is formed after a few minutes when normal blood diluted with water is treated with a solution of tannic and pyrogallic acids, whereas with blood having carbon monoxide in combination with the hemoglobin a light carmine suspension is formed; but in any mixture of normal blood and blood containing carbon monoxide the suspension will be a corresponding mixture of the two extremes of color. The apparatus consists of a set of standards to represent the different colors of varying but known amounts of carbon monoxide in combination with hemoglobin, to which unknown specimens can be matched and the amount of carbon monoxide hemoglobin evaluated. The percentage of saturation of carbon monoxide in the blood can easily be determined by this method to a degree of accuracy involving only 5 per cent error." The apparatus designed for this purpose is simple enough to be used without special training and it is durable and compact so that it can be carried in the pocket. The apparatus may be obtained from the Mine Safety Appliance Company, Pittsburgh.

AIR EMBOLISM FROM FILLING URINARY BLADDER WITH AIR. C. P. MATHÉ, J. d'urol. **28**:163, 1929.

Preliminary to operative removal of a vesical papilloma in a man, 56 years old, the bladder was being filled with air; a hissing sound was heard, and the patient died in collapse. After death, air bubbles were found in the iliac and mesenteric veins, in the abdominal part of the vena cava and in the renal vein. The air appears to have entered the circulation through ulceration in the neighborhood of the vesical tumor.

THE SEROLOGIC IDENTITY OF CARCINOMA. L. HIRSZFELD and W. HALBER, Klin. Wchnschr. **9**:918, 1930.

The authors sought by serologic studies to determine whether in carcinoma there are many antigens or only one carcinoma substance. They conclude that there is a definite serologic substance common to all; or at least there are many tumors, which probably is significant biologically and may be demonstrated in the serums of patients with carcinoma.

EDWIN F. HIRSCH.

LESIONS IN THE NERVOUS SYSTEM IN EXPERIMENTAL THALLIUM POISONING. R. GREVING and O. GAGEL, Zentralb. f. d. ges. Neurol. u. Psychiat. **120**:805, 1929.

Following an attempt at suicide by means of a rat poison containing thallium, a severe motor polyneuritis developed in a woman. Experiments were made and weakness and ataxia in hind extremities developed in dogs and cats. The nerves of the extremities presented marked degenerative changes.

ASCARIASIS THE CAUSE OF SUDDEN DEATH. W. LEWIŃSKI (Warsaw), Czas. lek. **6**:223, 1929.

Headache and vomiting suddenly developed in a girl, aged 9, She became unconscious and died in six hours. Autopsy revealed a mass of thirty-eight worms in the jejunum, 80 cm. from the pylorus. The jejunal mucous membrane was congested and swollen. The cause of death is ascribed to intoxication with ascaris poison.

Technical

THE CONTROLLED FLOCCULATION TEST IN THE DIAGNOSIS OF SYPHILIS. ALEXANDER MICHAILOFF, Am. J. Hyg. **11**:202, 1930.

The controlled flocculation test using the mastic-brain-muscle antigen has proved more accurate, more sensitive, more easily interpreted and more time-saving than the Wassermann or other flocculation tests. The brain-muscle antigen gives more sensitive and less false reactions when used in the regular Wassermann technic than do the antigens of Bordet-Ruelens and Bering-Werke (cholesterinized). In an accurate Wassermann test it is necessary to use at least three different antigens. Without the addition of complement our mastic-brain-muscle antigen gives a sensitive flocculation test. To avoid anticomplementary reactions, serums, after separation, should be shaken five minutes and then centrifugated and inactivated.

AUTHOR'S SUMMARY.

LEUCOCYTE COUNTS IN RABBITS. S. C. CHENG, Am. J. Hyg. **11**:449, 1930.

In the light of the observations recorded the following points especially must be taken into consideration in order to obtain consistent leukocyte counts in rabbits: (*a*) the age of the rabbit, (*b*) the time of day at which counts are made, (*c*) the manner of feeding, (*d*) pregnancy and the postparturient period, (*e*) the position of the animal and (*f*) the conditions of sensitization, infection or the carrier state, with *Bacillus bronchi-septicus* or *Bacterium lepisepticum*. With these factors eliminated or controlled, and with quiet animals, consistent counts can be obtained on successive days.

JOHN PHAIR.

THE EARLY DIAGNOSIS OF PREGNANCY, CHORIOEPITHELIOMA AND HYDATIDIFORM MOLE BY THE ASCHHEIM-ZONDEK TEST. S. ASCHHEIM, Am. J. Obst. & Gynec. **19**:335, 1930.

This test is based on the hormone from the anterior lobe of the hypophysis, which is increased in pregnant women and is found in abundance in the urine. The hormone activates the ovaries of the infantile mouse (aged 3 weeks and weighing 6 Gm.) to the production of mature follicles and corpora lutea, which in turn by the hormone they elaborate bring about estrus-like changes. The technic, briefly, consists in using six mice as specified which receive six hypodermic injections, three each, on the first and second days of the morning urine of the suspected pregnant woman. The single doses are: 0.2 and 0.25 cc. for the first two mice, 0.3 cc. for the third and fourth and 0.4 cc. for the fifth mouse. The sixth mouse acts as a control. One hundred hours after the first dose the mice are killed and the ovaries studied. The ovaries of control mice are pale grayish pink and hardly pinhead in size, while those of test mice are much larger and distinctly red and have submiliary yellowish protrusions corresponding to the corpora lutea. The microscopic test may verify this, and changes found as in estrus are seen in the uterus and vagina. This test has been found to be accurate in 98.6 per cent of 880 cases. The urine of women having hydatid moles or chorio-epitheliomas is strongly positive.

A. J. KOBAK.

A NEW TEST OF RENAL FUNCTION. R. T. BRAIN and H. D. KAY, Quart. J. Med. **22**:203, 1929.

The authors describe a glycerophosphate test in which 550 mg. of sodium glycerophosphate is given intravenously in a 50 per cent sterile solution, 10 cc. being given at one time. One hour after the test the bladder is emptied. The proteins are precipitated and phosphorus determinations are made. Before the test is given, a control specimen is obtained which gives the normal phosphorus excretion.

The difference between the control and the test specimen gives the excess phosphorus excretion. In normal persons 150 mg. of extra phosphorus is usually excreted. In renal impairment much lower values are obtained. In forty-four cases of renal disease this test has been compared with the urea concentration and the phenolsulphonphthalein test, and was found to be at least as valuable as these two tests, and in some instances more closely approached the clinical observations. The test is based on the enzymic activity of the kidney. The presence of phosphates in the kidney tissue has been held responsible for the inorganic phosphates of the urine. The authors had previously observed that in chronic nephritis and in experimental nephritis the phosphate content of the kidney was diminished, and the reduction in the enzymic activity seemed to be parallel with the degree of structural damage in the kidney.

N. Enzer.

The Demonstration of Tubercle Bacilli in Surgical Tuberculosis. M. Knorr and H. Friedrich, München. med. Wchnschr. **77**:173, 1930.

The diagnosis of surgical tuberculosis by means of animal inoculations was in error, at most, in 5 per cent. With intraglandular inoculation, the length of the test in 95 per cent was shortened to from ten to twenty-eight days without modifying the certainty of the result. Cultures made according to Hohn were negative in ten cases in which the animal tests were positive. Cultures were never positive when the animal inoculations were negative. Tubercle bacilli in material treated with sulphuric acid were demonstrated with greater ease by animal inoculation than by culture. Certain infections seemed to be caused by strains nonpathogenic for guinea-pigs. In these cases, attempts to grow the organisms were unsuccessful.

Authors' Summary.

The Phosphatide and Cerebroside Contents of the Spleen and Liver in Gaucher's Disease of Children, in Niemann-Pick's Disease, and Normally. Emil Epstein, Virchows Arch. f. path. Anat. **274**:294, 1929.

The figures for lecithin as given in the literature are far too high. If one extracts normal spleens with ether, working with fresh material and with formaldehyde-fixed material, the residue after evaporation of the ether is three times as heavy in the fresh material as in the fixed. It is different after extraction with alcohol; generally the fresh spleen leaves a residue which is only one-tenth heavier. Therefore, organs for lipoid extraction must be kept unfixed on ice, and, if possible, given to the chemist the same day. One can keep the organs for some time by slicing them and rubbing chemically pure sodium chloride into the slices.

Alfred Plaut.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

Annual Conversational Lecture, April 10, 1930

The Etiologic Grounds for Separating the Different Forms of Hypersensitiveness, with Special Reference to Anaphylaxis, Atopy (Hay-Fever—Asthma Group) and the Tuberculin Type. Arthur F. Coca.

One of the important contributions of the science of immunology is the paradoxic discovery that antibodies are sometimes the actual cause of disease.

The first instance of this discovery was in the demonstration by Landsteiner with Donath that paroxysmal hemoglobinuria is due to the presence of an antibody in the blood of these subjects, the autohemolysin, which causes the destruction of the red blood corpuscles if the blood has been chilled in the surface vessels to a temperature of 16 C. or lower.

Showing the Outstanding Differences Between Anaphylaxis and Atopy

	Anaphylaxis	Atopy
Antibodies	1. Produced by normal human being or animal.	1. Produced against usual atopens under normal conditions of contact only under atopic hereditary influence.
	2. Neutralize the antigen.	2. Usually incapable of neutralizing the antigen.
	3. Sensitize unstriped muscle of guinea-pig.	3. Do not sensitize unstriped muscle of guinea-pig.
	4. Do not sensitize human skin.	4. Sensitize human skin.
Shock-tissue	Normally susceptible to antibody-antigen reaction.	Normally insusceptible to antibody—antigen reaction.
Transmission to offspring	From female only; by passage of antibodies from mother's blood through the placenta.	From father or mother; by mendelian inheritance.
Desensitization	Complete in guinea-pig difficult in rabbit.	Impossible.

The second category of immunologic diseases is that of specific sensitiveness, comprising, in the human being, the hereditary conditions of asthma, hay-fever and eczema and also specific dermatitis (of that type known as dermatitis venenata) and serum disease, neither of which is subject to the special hereditary influence to which reference has been made.

In the accompanying table, the atopic (hereditary) hypersensitiveness of man is contrasted, as to its chief features, with the experimental form (anaphylaxis) in lower animals.

Since it has been found that under continuous contact with the excitant the age of the onset of symptoms of atopic sensitiveness is determined by heredity, the influence of contact in establishing this clinical condition must be entirely subordinate even among atopic persons. This is further emphasized by the fact that intensive natural or artificial contact, on the part of an atopic person, with an antigen to which he is not naturally sensitive does not render him atopically sensitive to that antigen.

The studies of Anderson and Schloss, of M. Walzer and of Ecker and of Cohen and Breitbart have shown that at least 90 per cent of normal human beings are constantly absorbing unaltered proteins through their mucous membranes; yet only those of atopic ancestry become clinically hypersensitive, and these usually to only a limited number of the absorbed proteins.

Furthermore, atopic hypersensitiveness is much less commonly exhibited to the "good" antigens, such as egg, milk and meat proteins, than to the "poor" antigens, such as the pollens.

All of these well established facts show the relative unimportance of contact in the etiology of the atopic form of hypersensitiveness; they refute the indirect attempts to identify atopy and anaphylaxis.

Book Reviews

REFLEX ACTION: A STUDY IN THE HISTORY OF PHYSIOLOGICAL PSYCHOLOGY. By FRANKLIN FEARING, Ph.D. Price, $6.50. Pp. 350, with illustrations. Baltimore: Williams & Wilkins Company, 1930.

This summary of the growth and present status of the concept of the reflex arc is timely, for it gives the historical perspective necessary for evaluating those recent movements in physiology and psychology that derive all human behavior from simple and conditioned reflexes, and for an appreciation of the reasons for a searching criticism of these movements. The history of the emergence from scholastic mysticism of the idea of the reflex as the natural operation of bodily mechanisms is outlined in the first seven chapters. These early efforts to find scientific formulation of the problem reached their logical fulfilment in la Mettrie's "Man a Machine," published in 1748, but at that time the factual knowledge of bodily organization was inadequate to support so sweeping a generalization.

At the close of the eighteenth century, the concept of reflex action as a type of neuromuscular response with certain specific objective characteristics was clearly formulated, despite the lack of adequate anatomic knowledge of the nervous structures involved. In the nineteenth century, this anatomic knowledge was so rapidly accumulated that there seemed to be reasonable hope of a satisfactory mechanistic account of all human behavior. Numberless specific reflexes were accurately investigated physiologically and their nervous pathways and centers were demonstrated.

Before the dawn of the twentieth century, the focus of interest shifted from the particular reflexes as units of behavior to the problem of their organization, their integration and their modifiability. The Russian school studied intensively the conditioning of reflexes (which is learning), and in Germany an "objective psychology" rapidly developed. The last mentioned movement culminated in the excesses of the American school of radical behaviorism. Meanwhile Sherrington, in 1906, published his "Integrative Action of the Nervous System," a work which, as one looks back on it, one recognizes as marking the turning point from an analytic toward a genuine and well founded synthetic treatment of the reflexes in their organic setting—not as isolated units of function, but as parts of the machinery of integration.

Today it is generally recognized that the "simple reflex" is a pure fiction, and there is a healthy skepticism regarding all of the traditional dogmas that have for so long been associated with the terms "reflex," "automatic," "involuntary" and "voluntary." It is concluded that reflexes can no longer "be regarded as isolated units of function in the intact nervous system," and it is equally clear, though not mentioned by our author, that in pathologic processes the local reflexes, though valuable diagnostic signs, must be interpreted critically in their relation to the organization as a whole. Their disorders may be far more instructive as indicators of systemic disturbances than merely as aids in the localization of lesions.

The swing at the present time is strongly away from regarding the reflex as a practicable unit of behavior. In psychology, this is most evident in the so-called Gestalt movement. "From the point of view of physiological psychology, we are concerned with the reflex act as part of the total response pattern of the functioning organism, rather than with the analysis of the functional components of the isolated reflex arc." This is as true in physiology as in psychology, and the author has missed an instructive exemplification of it, for he has overlooked the work of Coghill ("Anatomy and the Problem of Behavior," Cambridge University Press, 1929; and earlier works there cited), who has shown that local reflexes, so far from being primary elements of behavior, are secondary to total patterns

of behavior and are derived from these. They are partial patterns which in ontogeny are slowly emancipated from mass movements of the whole bodily musculature. Adult behavior patterns are not fabricated from an assemblage of separate reflexes, but all bodily activities are perfectly integrated from the beginning of embryonic development and the local reflexes come at the close of the developmental process, not at its beginning. This reversal of our traditional reflexology is well authenticated by observation and experiment. It may have important applications in medical practice, but this field awaits exploration.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1928-1929. Price, 3 shillings. Pp. 153. London: His Majesty's Stationery Office, 1930.

The work of the Medical Research Council is supported by public and by private funds and is conducted by its own full-time staff in the National Institute for Medical Research at Hampstead; by so-called clinical units in various hospitals in London and elsewhere, and by grants-in-aid to workers in various centers for research on a great variety of problems. In the introduction to the detailed report certain major topics are discussed and special attention may be called to the section dealing with clinical research and experimental medicine. Here is discussed again the questions whether there is a science of experimental medicine of which the actual material for study is the human patient or is scientific work by the physician or surgeon limited to the application in his art of scientific results worked out elsewhere in the laboratory and delivered to him for use? The Council makes it clear that all in its power will be done to encourage further the direct scientific study of disease in man by recruiting "young workers of ability who are prepared to test themselves in this branch of medical research with the view to its becoming their life work." This section bears directly on our problem of full-time clinical teachers. Without going into greater detail, let it be said that the report conveys an excellent and instructive view of the scope and nature of the activities of the Medical Research Council, which is the most important factor by way of organized effort in medical research in Great Britain. The report is a model of its kind; it should be copied by similar agencies elsewhere, and it should be studied by all who are concerned in the promotion of medical research and the practical application of results.

PRAKTIKUM DER GEWEBEPFLEGE ODER EXPLANTATION BESONDERS DER GEWEBEZÜCHTUNG. By RHODA ERDMANN. Second edition. Price, 14.80 marks. Pp. 148, with 99 illustrations. Berlin: Julius Springer, 1930.

Although the author considers this merely a handbook for a practical course for students, it must also be ranked as an excellent guide for investigators in the field of tissue culture. The author defines her terms carefully and calls timely attention to the errors of nomenclature and misuse of terms that are gradually creeping into the subject. A good example is the expression "generations" of cultures when what is really meant is a series of "transfers" of part of a tissue grown "in vitro." Full details of the technic for the explanation and care of various tissues of animals and man are given. A feature of the book is the detailed description of the best methods for staining whole explants or sections of them and for the differentiation of the various types of tissue and cell. The illustrations are well chosen and of good quality. There are practically no errors of printing, and such a discrepancy as "Typhusbakterien" in the legend of figure 93, page 128, and "Tuberkelbacillen" in the text referring to this illustration was the only one noted by the reviewer. The book is not merely a technical manual, for it contains a discussion of the value and limitations of this type of investigation, brief but good descriptions of the historical background of every phase of the subject, summaries of the established facts, and, what is more important, suggestions for future investigations.

BACTERIAL METABOLISM. By MARJORY STEPHENSON, M.A., Associate of Newnham College, Cambridge; Member of the Scientific Staff of the Medical Research Council. Price, $7.00. Pp. 320, with diagrams. New York: Longmans, Green & Company, 1930.

This volume of two hundred and seventy text pages is intended, according to the preface, to "choose from the mass of data on the chemical activities of bacteria facts — to appraise our knowledge of bacteria as living organisms apart from their rôle as disease germs or the bearers of commercially important catalysts." The point of view is mathematic rather than biologic. Emphasis appears to be placed on isolated, unrelated details rather than on coordination and unification of the material considered. The first seven chapters, comprising an introduction and sections on energy relations and fermentation, respiration, growth and nutrition, carbohydrate breakdown, "viscous fermentation" and protein breakdown, are chiefly compilations, gleaned from many sources, illustrative of the versatility of bacteria and related forms under various conditions rather than informative concerning their biology. The last two chapters, however, which make up one fifth of the book, on nitrogen fixation and autotrophic bacteria, are well organized, constructive in their scope and more in harmony with the title.

Books Received

DIE GASBEHANDLUNG BÖSARTIGER GESCHWÜLSTE. Von Dr. Bernhard Fischer-Wasels, O. Ö. Professor der allgemeinen Pathologie und pathologischen Anatomie an der Universität, Direktor des Senckenbergischen Pathologischen Instituts zu Frankfurt am Main. Unter mitwirkung von Privatdozent Dr. W. Büngeler, Dr. J. Heeren, Dr. S. Heinsheimer, Dr. G. Joos. Mit 82 zum teilfarbigen Abbildungen im Text und zahlreichen Tabellen. Price, 65 marks. Pp. 472, with 82 illustrations. Munich: J. F. Bergmann, 1930.

PRAKTIKUM DER GEWEBEPFLEGE ODER EXPLANTATION BESONDERS DER GEWEBEZÜCHTUNG. Von Rhoda Erdmann. Zweite Auflage. Price, 14.80 marks. Pp. 148, with 99 illustrations. Berlin: Julius Springer, 1930.

REPORT OF THE LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA. By Herbert Fox, M.D., Pathologist. Pp. 60. Philadelphia, 1930.

CLINICAL ATLAS OF BLOOD DISEASES. By A. Piney, M.D., M.R.C.P., Research Pathologist, Cancer Hospital, London, and Consulting Pathologist, Chelmsford Hospital and Stanley Wyard, M.D., M.R.C.P., Physician, Bolingbroke Hospital, and Assistant Physician, Cancer Hospital, London. Price, $4. Pp. 98, with 36 illustrations. Philadelphia: P. Blakiston's Son & Company, 1930.

TEXT BOOK OF PATHOLOGY INCLUDING BACTERIOLOGY, ANIMAL PARASITOLOGY, LABORATORY METHODS AND LABORATORY DIAGNOSIS OF DISEASES. By Dhirendra Nath Banerjee M.B. (Cal.), M.D. (Berlin), Demonstrator of Pathology, Carmichael Medical College; Radiologist, Chittaranjan Hospital; Radiologist, Calcutta Polyclinic, Ltd.; Author of "Cholera and Its Modern Treatment." Second edition, revised and enlarged. Price, $4. Pp. 646, with 314 illustrations. Calcutta: The Medical Bureau, 1929.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1928-1929. Price, 3 shillings, net. Pp. 153. London: His Majesty's Stationery Office, 1930.

OM OPRINNELSEN TIL OG UTVIKLINGEN AV DE TUBERKULOSE SYKDOMMER. TUBERKULOSENS ETIOLOGI, PATOGENESE OG PATOLOGISKE ANATOMI [On the Origin and Development of the Tuberculous Diseases. The Etiology, Genesis and Pathologic Anatomy of Tuberculosis]. En oversikt for laeger og studerende. Av Francis Harbitz, Professor, dr. med. Pp. 170, with 33 illustrations. Oslo: H. Aschehoug & Company, 1930.